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ENDOMETRIOSIS-ASSOCIATED INFERTILITY: THE ROLE OF HORMONES AND ITS CORRECTION

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Background. *Endometriosis-associated infertility (EAI) has a number of specific features, which are crucial in the choice of medical treatment.*

Objective. *The aim of the study is to analyze endocrine profile in women with EAI before and after sclerotherapy and pregravid preparation (PP), which includes a vitamin complex FT 500 plus with inositol and vitamin D3.*

Methods. *The study involved 70 women aged 21-40 years with endometriosis-associated infertility. The comparison group included 30 women with tuboperitoneal infertility. ELISA was used to determine concentrations of Anti-Mullerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and estradiol in blood serum using a standard kit by Diagnostic Systems Laboratories, Inc (USA). During two menstrual cycles the FT 500 plus was prescribed once a day from the 2^{nd}/3^{rd} day of the cycle, vitamin D3 was prescribed at the dose of 2,000 IU for women without its deficiency and in therapeutic doses in case of hypovitaminosis. The sclerotherapy with 95% ethanol solution was performed on the 6^{th}-8^{th} day of menstrual cycle.*

Results. *It was established that in women with EAI undergoing PP and sclerotherapy the level of AMH was lower (by 12.90%) as well as progesterone (by 9.84%), while FSH (by 14.47%), LH (by 21.14%) and estradiol (by 35.55%) was higher compare to the comparison group. At the same time, FSH (by 21.98%), LH (by 32.89%) and estradiol (by 32.23%) concentrations were significantly lower compare to their primary indices before sclerotherapy.*

Conclusions. *Sclerotherapy and PP with a vitamin complex, inositol and vitamin D3 has a positive effect on endocrine profile in women with endometriosis-associated infertility*

KEYWORDS: **infertility; endometriosis; hormones; sclerotherapy; inositol; vitamin D3.**

Introduction

Genital endometriosis is one of the most common diseases in women of reproductive age. It attracts attention of scientists and practitioners around the world. According to the literature, every tenth woman of reproductive age suffers from endometriosis that is 176 million women (World Population Projection Tables by Country and Group, 2010). The main symptoms of endometriosis are infertility, menorrhagia, dysmenorrhea, dyspareunia, chronic pelvic pain. In 20-25% of women, this disease is asymptomatic [1].

Endometriosis-associated infertility is characterized by a number of specific features that must be considered when choosing treatment methods. In each case, treatment should be individual, taking into account all clinical factors, as well as the impact of the disease and the effect of treatment on the quality of woman's life [2, 3].

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The classic surgical treatment of endometriosis is necessary in cases of cyst capsule; according to the literature, it facilitates the lowest number of recurrences and the highest number of clinical pregnancies [4-7]. However, it should be taken into account that besides the capsule, a significant part of healthy ovarian tissue with antral follicles is removed [8]. Therefore, the search for less traumatic and more effective treatment, i.e. puncture of endometriomas and sclerotherapy, is carried on [9].

To date, the choice of treatment for women with endometriosis-associated infertility for assisted reproductive technology (ART) programs is still controversial. According to evidence-based medicine it is established that in women with endometriosis-associated infertility, the balance between intracellular trace elements is disturbed; free radical oxidation processes are activated, which leads to oxidative stress, which consecutively may disturb the effectiveness of ART. Reactive oxygen species are usually significant in a number of reproductive tract functions, but their overproduction has a negative effect on estrogen levels, altering

steroidogenesis, thereby preventing oocyte maturation and ovulation [10, 11]. Therefore, we consider using antioxidants during pregravid preparation of patients with endometriosis-associated infertility before COS protocol reasonable.

The aim of the study was to analyze concentrations of the reproductive hormones in women with endometriosis-associated infertility before and after sclerotherapy and pregravid preparation with a complex vitamin medication comprising inositol and vitamin D3.

Methods

The study involved 70 women aged 21-40 years with endometriosis-associated infertility, who were treated in the Medical Center "Clinic of Professor Stefan Khmil" in 2015-2020. The comparison group included 30 women of the same age with tuboperitoneal infertility, diagnosed by laparoscopy or echosalpingography. Exclusion criteria were for patients with endometriosis, stage 3-4, polycystic ovary syndrome, uterine fibroids (submucosal, symptomatic subserosal or intramural with a diameter of more than 2 cm), who were not included into the study groups. External genital endometriosis was verified by laparoscopy and confirmed by histopathological examination (endometrioid cyst of an ovary or both ovaries).

The patients with endometriosis-associated infertility were divided into 2 groups. Group 1 (n=34) involved women of reproductive age (21-40 years old) with endometrioid cysts of up to 6.5 cm in diameter, who underwent sclerotherapy and pregravid preparation with a complex vitamin medication comprising inositol and vitamin D3; group 2 (n=36) comprised women, who underwent sclerotherapy. The presence of cysts was confirmed by ultrasound and bimanual examination.

Determination of hormone indicators was performed in different phases of the cycle before and after sclerotherapy and pregravid treatment. Determination of concentrations of reproductive hormones was performed in the certified laboratory of the Medical Center "Clinic of Professor Stefan Khmil". ELISA was used to determine the concentrations of Anti-Mullerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and estradiol (E2) in blood serum according to manufacturer's instructions using a standard set of reagents provided by Diagnostic Systems Laboratories, Inc. (USA), by means of the StatFax analyzer.

The vitamin complex FT 500 plus for women of group 1 was prescribed at the dose of 1 sachet once a day from the 2nd or 3rd day of the cycle, after taking blood test for hormones, i.e. AMH, FSH and LH, during 2 menstrual cycles (cycle in which sclerotherapy was performed and subsequent cycle). Vitamin D3 was prescribed at prophylactic doses of 2,000 IU to those patients who were not diagnosed with a deficiency of this vitamin and at medium therapeutic doses in cases of hypovitaminosis during 2 menstrual cycles.

The procedure of sclerotherapy was performed in a sterile surgery room with or without general anesthesia on the 6th-8th day of the menstrual cycle by transvaginal puncture of the cyst and aspiration of its contents under the control of transvaginal ultrasound, introduction of sclerosing solution into the cyst capsule (without violating its integrity). 76 % ethanol solution was used as a sclerosant installed in a volume of 50%-90% of the cyst size under ultrasound control with its subsequent aspiration.

Statistical analysis of the results was performed using Microsoft Office Excel and Statistica 7.0 software. The choice of the data analysis method was based on the number of the groups, their distribution, as well as the equality of variances. All data were verified by the one sample Kolmogorov-Smirnov test to confirm normality. Abnormal distribution data was analyzed by a nonparametric test (the Mann-Whitney U test and the Kruskal-Wallis test). The indicators with abnormal distribution were defined as Me (Q25; Q75) (medians and Q25 and Q75 quartiles). The biochemical findings were analyzed by repeated non-parametric ANOVA test for multiple comparisons. A probability level of less than 0.05 was considered to be statistically significant.

Results

Study of the reproductive hormones of the women with endometriosis-associated infertility proved a significantly lower level of Anti-Mullerian hormone (AMH) in women before and after sclerotherapy compare to the comparison group. It was established that sclerotherapy in women with endometriosis-associated infertility caused a decrease of FSH by 20.31% compare to the indices before the therapy for endometriomas but was still statistically significantly higher compare to the comparison group (by 20.75%).

Also, a decrease of the LH concentration by 16.96% was evidenced in the patients after

sclerotherapy compare to the indices before the therapy for endometriomas. It was statistically significantly higher than those of the comparison group (by 39.02%). The level of estradiol in blood serum of the women with endometriosis-associated infertility after sclerotherapy was significantly lower by 26.63% compared to that before surgery, but was still significantly higher compare to the comparison group (by 41.11%). Similar changes were observed for progesterone levels on the 19th-21st day of menstrual cycle (d.m.c.) in blood serum of the women with endometriosis-associated infertility after sclerotherapy; specifically, it increased by 14.94% compare to the indices before sclerotherapy, but was by 25.11% lower compare to the comparison group. Please note that sclerotherapy did not affect the level of prolactin (Table 1).

The women with endometriosis-associated infertility, who underwent pregravid preparation with a complex vitamin medication comprising inositol and vitamin D3 as well as sclerotherapy had significantly lower levels of AMH (by 12.90%), progesterone (by 9.84%) and significantly higher levels of FSH (by 14.47%), LH (by 21.14%) estradiol (by 35.55%) compare to the comparison group. At the same time, significantly lower levels of FSH (by 21.98%), LH (by 32.89%) and estradiol (by 32.23%) were evidenced compare to those before sclerotherapy (Table 2).

Table 1. Concentration of reproductive hormones in the women with endometriosis-associated infertility before and after sclerotherapy and no pregravid preparation (Me (Q25; Q75))

Indicators	Comparison group (n=30)	Before sclerotherapy (n=36)	After sclerotherapy (n=36)
AMH, pmoL/L	1.75 (1.56; 1.92)	1.50* (1.30; 1.73)	1.50* (1.30; 1.73)
FSH, IU/L	7.95 (6.93; 8.50)	11.55* (10.90; 12.43)	9.60* (8.78; 10.60)
LH, IU/L	6.15 (5.45; 6.80)	10.00* (9.30; 10.70)	8.55* (7.98; 9.23)
Estradiol on the 2nd-3rd d.m.c., pmoL/L	36.85 (33.70; 40.08)	65.85* (60.40; 73.90)	52.00* (48.63; 55.53)
Progesterone on the 19th-21st d.m.c. nmol/L	13.95 (12.63; 15.58)	9.70* (8.18; 11.43)	11.15* (9.63; 12.23)
Prolactin, ng/mL	10.95 (10.40; 12.28)	10.40 (9.60; 11.40)	10.30 (9.50; 11.08)

*Notes. Statistically significant difference (p<0.05) compared to: * – the comparison group. Me (Q25; Q75) (median, Q25 and Q75 quartiles).*

Table 2. Concentration of reproductive hormones in the women with endometriosis-associated infertility before and after sclerotherapy, who underwent pregravid preparation with a complex vitamin medication comprising inositol and vitamin D3 (Me (Q25; Q75))

Indicators	Comparison group (n=30)	Before sclerotherapy (n=34)	After sclerotherapy (n=34)
AMH, pmoL/L	1.75 (1.56; 1,92)	1.55* (1.40; 1.78)	1.50* (1.30; 1.80)
FSH, IU/L	7.95 (6.93; 8.50)	11.10* (10.50; 12.40)	9.10* (8.30; 9.88)
LH, IU/L	6,15 (5.45; 6.80)	9.90* (8.65; 11.00)	7.45*# (6.93; 8.65)
Estradiol on the 2nd-3rd d.m.c., pmoL/L	36.85 (33.70; 40.08)	66.05* (61.50; 74.10)	49.95* (43.73; 55.38)
Progesterone on the 19th-21st d.m.c. nmol/L	13.95 (12.63; 15.58)	10,45* (8.95; 12.48)	12.70*# (11.13; 13.50)
Prolactin, ng/mL	10.95 (10.40; 12.28)	11.00 (9.53; 12.18)	10.80 (10.00; 12.00)

*Note. Statistically significant difference (p<0.05) compared to: * – the comparison group, # – data before sclerotherapy. Me (Q25; Q75) (median, Q25 and Q75 quartiles).*

In order to compare the data, we analyzed the effect of pregravid preparation on the concentration of reproductive hormones in women with endometriosis-associated infertility after sclerotherapy, so the value of hormones before this surgical manipulation, we considered 100%. It was found that the use of a complex vitamin medication comprising inositol, as well as vitamin D3 caused a decrease of LH level (by 10.25%), as well as an increase of progesterone level (by 6.58%).

Discussion

The results of the study proved a positive effect of sclerotherapy on the endocrine profile of women with endometriosis-associated infertility. According to the literature, sclerotherapy of endometriomas is recommended for women with recurrent endometrioid cysts [12] to preserve the ovarian reserve avoiding surgical excision [13]. It was established that the recurrence rate after sclerotherapy ranged from 12.9% to 20% that almost did not differ from the indicators after laparoscopic cystectomy [14, 15].

It was found out that the number of antral follicles was increasing in the patients who underwent sclerotherapy for recurrent endometriomas [16, 17]. The AMH blood level was an indirect marker of ovarian reserve. In our study, there were no significant changes in the AMH level in blood serum before and after sclerotherapy that is consistent with other studies [18]. Sclerotherapy can improve ovarian blood supply and follicle development [16], which consecutively may increase the probability of future fertility in the women undergoing sclerotherapy. The effect of sclerotherapy on the reproductive hormones level is still doubtful. Thus, Wang Xiaotao et al. established decreased levels of E2, FSH and LH [19]. In a study of Saeed Alborzi et al., the effect of sclerotherapy was associated with ovarian activity; in particular, a decrease of the FSH level as well as an increase of the AMH level and antral follicles [20]. On the other hand, Aflatoonian A et al. did not prove any significant difference between the studied hormones (FSH, LH) before and after sclerotherapy. The difference of the results may be due to different sizes of cysts in the studied female patients (our study involved women with endometrioid cysts of up to 6.5 cm in diameter), presence of cystectomy in medical history, localization (unilateral or bilateral endometrioma), and recurrence of endometrioid cysts.

Pregravid preparation with a complex vitamin medication comprising inositol and vitamin D3 had an effect. Inositol is a component of phospholipids and is a part of the cytoplasmic membranes as a phosphoinositide. Inositol binds Ca^{2+} channels and stimulates intracellular release of Ca^{2+}. Increased intracellular Ca^{2+} level is significant for oocyte maturation, fertilization, and embryonic development. A sufficient concentration of inositol in the follicular fluid reduces the free radicals' level and membrane protein damage and increases the number of good quality oocytes [21]. 1.25-dihydroxy-vitamin-D3 (vitamin D), except calcium-phosphate homeostasis, has a significant immunomodulatory effect (affects the local immune environment in an autocrine/paracrine way), affects the processes of cell proliferation and differentiation. Studies have shown that vitamin D reduces concentration of anti-inflammatory cytokines: IL-6, interferon-g, IL-2, and TNF-a [21]. The combination of vitamin D3 multivitamin complex comprising inositol leads to normal ovarian activity, improves metabolic and hormonal profile, in particular, controls increased level of luteinizing hormone and oxidative stress [22].

Conclusions

Sclerotherapy and pregravid preparation with a complex vitamin medication comprising inositol and vitamin D3 has a positive effect on the endocrine profile in women with endometriosis-associated infertility.

Conflicts of Interest

Authors declare no conflict of interest.

Authors' Contributions

Iryna Kulyk – investigation, conceptualization, data curation, formal analysis, writing – original draft.

Stefan Khmil – data curation, writing – reviewing and editing.

ОСОБЛИВОСТІ ГОРМОНАЛЬНОГО ФОНУ ЖІНОК З БЕЗПЛІДДЯМ НА ФОНІ ЕНДОМЕТРІОЗУ ТА МЕТОДИ ЇХ КОРЕКЦІЇ

І.І. Кулик, С.В. Хміль
ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Безпліддя на фоні генітального ендометріозу характеризується низкою специфічних особливостей, які необхідно врахувати при виборі методів лікування.*

Мета дослідження – *проаналізувати концентрацію гормонів репродуктивної системи у жінок з безпліддям на фоні ендометріозу до та після склеротерапії із застосуванням прегравідарної підготовки комплексним вітамінним препаратом з інозитолом та вітаміном D3.*

Методи дослідження. *У дослідження увійшло 70 жінок віком від 21 до 40 років з ендометріоз-асоційованим безпліддям та 30 жінок з трубно–перитонеальним фактором, як група порівняння. Визначення концентрації гормонів (антимюллерового гормону (АМГ), фолоікулостимулюючого гормону (ФСГ, лютеїнізуючий гормон (ЛГ), прогестерон та естрадіол) проводили за допомогою ІФА з використанням наборів «Diagnostic Systems Laboratories, Inc.» (США) на аналізаторі «StatFax».*

Препарат FT 500 plus для жінок 1групи призначали в дозуванні 1 саше – 1 раз в день з 2-го або 3-го дня циклу протягом 2-х менструальних циклів (цикл у якому проводилась склеро терапія та наступний цикл). Вітамін D3 призначався у профілактичних дозах 2000 ОД жінкам, у яких не було діагностовано дефіциту даного вітаміну та в середньотерапевтичних дозах при гіповітамінозі протягом 2-х менструальних циклів. Процедура склеротерапії проводилась в умовах стерильної операційної на 6–8-й день менструального циклу шляхом трансвагінальної пункції кісти та аспірації її вмісту під контролем трансвагінального УЗД, введення в капсулу кісти 95 % розчину етанолу.

Результати. *У жінок з безпліддям на фоні ендометріозу, яким призначали прегравідарну підготовку комплексним вітамінним препаратом з інозитолом та вітаміном D3 та проводили склеротерапію, встановлено вірогідно нижчий рівень АМГ (на 12,90 %) та прогестерону (на 9,84 %) і вірогідно вищий рівень ФСГ (на 14,47 %), ЛГ (на 21,14 %) та естрадіолу (на 35,55 %) відносно групи порівняння. При цьому встановлено вірогідно нижчі показники ФСГ (на 21,98 %), ЛГ (на 32,89 %) та естрадіолу (на 32,23 %) проти їх значень до проведення склеротерапії.*

Висновки. *У кожному конкретному випадку лікування має бути індивідуальним, повністю враховувати всі клінічні фактори, а також вплив захворювання та ефект лікування на якість життя жінки. Проведення склеротерапії із застосуванням прегравідарної підготовки комплексним вітамінним препаратом з інозитолом та вітаміном D3 у жінок з ендометріоз-асоційованим безпліддям має позитивний вплив на гормональний фон.*

КЛЮЧОВІ СЛОВА: **безпліддя; ендометріоз; гормони; склеротерапія; інозитом; вітамін D3.**

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References

1. Abbas S, Ihle P, Koster I, Schubert I. Prevalence and incidence of diagnosed endometriosis and risk of endometriosis in patients with endometriosis-related symptoms: findings from a statutory health insurance-based cohort in Germany. Eur J Obstet Gynecol Reprod Biol 2012;160:79–83.

2. Giudice LC. Clinical Practice: Endometriosis. N Engl J Med 2010; 362: 2389-98.

DOI: https://doi.org/10.1056/NEJMcp1000274

3. Bulun SE. Endometriosis. N Engl J Med 2009;360(3): 268-79.

DOI: https://doi.org/10.1056/NEJMra0804690

4. Shestakova IG, Ipastova ID. [Endometriosis: a new consensus — new solutions: Global consensus on the management of patients with endometriosis as a first step towards the creation of industry standards: News bulletin]. Moscow: Status Praesens, 2014; 16 p. (In Russian).

5. Shcherbyna NA, Demidenko DI, Demidenko AD. [Melatonin's tocolytic activity in threatening preterm birth]. Emergency Medicine. 2014;5:100-102. (In Russian).

6. Borham MM. Comparison between omentoplasty and partial cystectomy and drainage (PCD) techenques in surgical management of hydatid cysts liver in endemic area (Yemen). J. Egypt. Soc. Parasitol. (JESP) 2014; 44(1):145-50.

DOI: https://doi.org/10.12816/0006454

7. Shakiba K, Bena JF, M McGill K. Surgical treatment of endometriosis: a 7-year follow-up on the requirement for further surgery. Obstetrics and Gynecology. 2008;111(6):1285-92.

https://doi.org/10.1097/AOG.0b013e3181758ec6

8. Khamoshina MB, Vakhabova MI, Kalinina EA. [Pharmacotherapy of endometriosis: opportunities and perspectives]. Meditsnskyi Sovet. 2013;8: 23-7. (In Russian).

9. Matsuzaki S, Houlle C, Darcha C. Analysis of risk factors for the removal of normal ovarian tissue during laparoscopic cystectomy for ovarian endometriosis. Human Reproduction. 2009; 24(6):1402-06.

DOI: https://doi.org/10.1093/humrep/dep043

10. Singh AK, Chattopadhyay R, Chakravarty B, Chaudhury K. Markers of oxidative stress in follicular fluid of women with endometriosis and tubal infertility undergoing IVF. Reprod Toxicol. 2013; 42:116-24.

DOI: https://doi.org/10.1016/j.reprotox.2013.08.005

11. Augoulea A, Mastorakos G, Lambrinoudaki I, Christodoulakos G, Creatsas G. The role of the oxidative-stress in the endometriosis-related infertility. Gynecol Endocrinol.2009; 25: 75-81.

DOI: https://doi.org/10.3917/eres.strei.2009.01.0075

12. Rubod C, Jean Dit Gautier E, Yazbeck C. Surgical management of endometrioma: Different alternatives in term of pain, fertility and recurrence. CNGOF-HAS Endometriosis Guidelines. Gynecol Obstet Fertil Senol. 2018 Mar;46(3):278–89.

13. Miquel L, Preaubert L, Gnisci A, Netter A, Courbiere B, Agostini. Transvaginal ethanol sclerotherapy for an endometrioma in 10 steps. Fertility and Sterility. 2020.Oct;115(1):259-64.

DOI: https://doi.org/10.1016/j.fertnstert.2020.08.1422

14. Gatta G, Parlato V, Di Grezia G, Porto A, Cappabianca S, Grassi R. Ultrasound-guided aspiration and ethanol sclerotherapy for treating endometrial cysts. Radiol Med.Torino. 2010 Dec;115(8): 1330–9.

DOI: https://doi.org/10.1007/s11547-010-0586-0

15. Aflatoonian A, Rahmani E, Rahsepar M. Assessing the efficacy of aspiration and ethanol injection in recurrent endometrioma before IVF cycle: A randomized clinical trial. Iran J Reprod Med. 2013 Mar;11(3):179–84.

16. Fisch JD, Sher G. Sclerotherapy with 5% tetracycline is a simple alternative to potentially complex surgical treatment of ovarian endometriomas before in vitro fertilization. Fertil Steril. 2004;82(2):437-41.

DOI: https://doi.org/10.1016/j.fertnstert.2004.01.031

17. Tsoumpou I, Kyrgiou M, Gelbaya TA, Nardo LG. The effect of surgical treatment for endometrioma on in vitro fertilization outcomes: a systematic review and meta-analysis. Fertil Steril. 2009;92(1):75-87.

DOI: https://doi.org/10.1016/j.fertnstert.2008.05.049

18. André GM, Vilarino FL, Christofolini DM, Bianco B, Barbosa CP. Aspiration and ethanol sclerotherapy to treat recurrent ovarian endometriomas prior to in vitro fertilization–a pilot study. Einstein (Sao Paulo). 2011 Dec;9(4):494-8.

DOI: https://doi.org/10.1590/s1679-45082011ao2081

19. Wang XT, Meng B, Liu JF, Jin FX and Xu H. The influences of ultrasound guided radiofrequency ablation assisted by polyol on fibroid volume and endocrine function among patients with uterine fibroid. Modern Practical Medicine 2018; 30: 1425-28.

https://doi.org/10.1093/rfs/hhx014

20. Alborzi S, Namavar Jahromi B, Ahmadbeigi M. Recovery Rate of Patients with Recurrent Ovarian Endometriomas Using Sclerotherapy with 95% Ethanol. Journal of Obstetrics, Gynecology and Cancer Research (JOGCR). 2018 Sep 10;3(3):105-10.

DOI: https://doi.org/10.1002/epi4.12285

21. Khmil SV, Kulyk II, Mykula RP. [Antioxidant therapy in women with infertility caused by endometriosis]. Bulletin of social hygiene and health care organization. 2018; 4 (78):56-63 (In Ukrainian).

DOI: https://doi.org/10.11603/1681-2786.2018.4.10031

22. Tsysar Yv, Andriets OA. [The influence of pathology of thyroid gland on menstrual function in girls of pubertal age]. Bukovinian Medical Bulletin. 2011;15(2):130–4. (In Ukrainian).

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PLEURAL ENDOMETRIOSIS: BLOODY PLEURAL EFFUSION IN A 28-YEAR-OLD FEMALE WITH PRIMARY INFERTILITY (CASE REPORT)

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Background. *Endometriosis is defined as presence of endometrial glands outside the uterine cavity and it most commonly involves the structures within the pelvic cavity. Thoracic endometriosis syndrome usually presents as pneumothorax, hemoptysis, hemothorax or pulmonary nodules. Endometriosis presenting as hemorrhagic pleural effusion is rarely reported.*

Objectives. *The aim of the study was to describe pleural endometriosis presenting as hemorrhagic pleural effusion and to insist on the role of medical thoracoscopy in making the diagnosis with the help of a case report.*

Methods. *A case report of pleural endometriosis as a non-resolving hemorrhagic pleural effusion is presented.*

Results. *A 28-years old female on treatment for her primary infertility presented with non-resolving bloody pleural effusion and she was on empirical anti-tubercular drugs for the last four months. Medical thoracoscopy revealed flat brownish grey plaques over the diaphragmatic pleura and the histology of pleural tissue revealed pleural endometriosis. She was initiated on gonadotropin releasing hormone-leuprolide and there was some clinico-radiological improvement.*

Conclusions. *Thoracic endometriosis should be considered as one of the differentials for pleural effusion in female patient especially on treatment for infertility. Medical thoracoscopy should be the investigation of choice for evaluating pleural effusions before initiating empirical treatments.*

KEYWORDS: **pleural endometriosis; catamenial pleural effusion; thoracic endometriosis syndrome; hemorrhagic pleural effusion.**

Introduction

Endometriosis is defined as presence of endometrial glands outside the uterine cavity and it most commonly involves the structures within the pelvic cavity. Thoracic endometriosis syndrome usually presents as pneumothorax, hemoptysis, hemothorax or pulmonary nodules. Pleural endometriosis is characterized by presence of functional endometrial tissue in the pleura [1]. Malignancy and tuberculosis are common causes of hemorrhagic pleural effusion. Endometriosis presenting as hemorrhagic pleural effusion are rarely reported [2]. Here we report a case of this rare entity in a 28-years old female.

Case Report

A 28-years old non-smoker female patient was referred to our department with moderate pleural collection after presenting to emergency department with cough and exertional breathlessness. Her cough was dry in nature and her effort tolerance was around 500 meters. There was no history of fever, chest pain, hemoptysis, loss of appetite and weight loss. The patient had significant past history, as she was being treated for her primary infertility for the last two years and has received multiple doses of clomiphene citrate.

As she failed in medical management, she was advised intrauterine insemination. During the work up for the above she was incidentally diagnosed to have right moderate pleural collection. Diagnostic thoracocentesis revealed straw colored exudative lymphocytic fluid, ultrasound guided pleural biopsy was done with Abram needle and the histopathology revealed lymphohistiocytic infiltrates. Based on the above results she was initiated on four anti-tubercular drugs of rifampicin, isoniazid, ethambutol and pyrazinamide according to body weight.

She attained menarche at the age of seventeen and had dysmenorrhea in her each cycle, which lasts for 8-10 days. She had no past history of diabetes, hypertension, asthma or tuberculosis. There was no history suggestive of connective tissue disease. Patient was born from a non-consanguineous marriage; both

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her parents and siblings were healthy. Her general examination, cardiovascular, abdominal and neurological system examinations were unremarkable. Respiratory examination revealed reduced chest expansion, stony dull note and reduced breath sounds in right mammary, interscapular and infrascapular areas.

Her blood investigations revealed hemoglobin of 12.8 gm/dL, platelets of 2,86,000/μL and total leukocyte count 8,500/μL with differentials (neutrophil 62%, lymphocyte 37%, and eosinophils 1%). She had normal renal and liver function (protein and albumin 6.4 and 4.5 g/dL respectively) tests. Her sputum smears were negative for acid fast bacilli. Underlying connective tissue and vasculitis were ruled out by appropriate investigations. Admission chest X ray showed moderate pleural effusion on the right side and computed tomography revealed normal lung parenchyma with moderate pleural effusion on the right side.

Diagnostic thoracocentesis revealed hemorrhagic lymphocytic exudative pleural effusion (total cell count: 480/μL mainly lymphocytic [78%]); proteins: 4.5g/dL; glucose: 107 mg/dL; LDH: 455 IU/L; adenosine deaminase: 18.6 U/L). Pleural fluid gram stain, tubercular cultures were sterile and cytological examination was negative for malignant cells. Bronchcoscopy done ruled out endobronchial lesions. Medical thoracoscopy revealed normal visceral pleura, minimal adhesions in the posterior costophrenic recess and flat brownish gray plaques with hematoma were noticed in parietal pleura (Fig. 1).

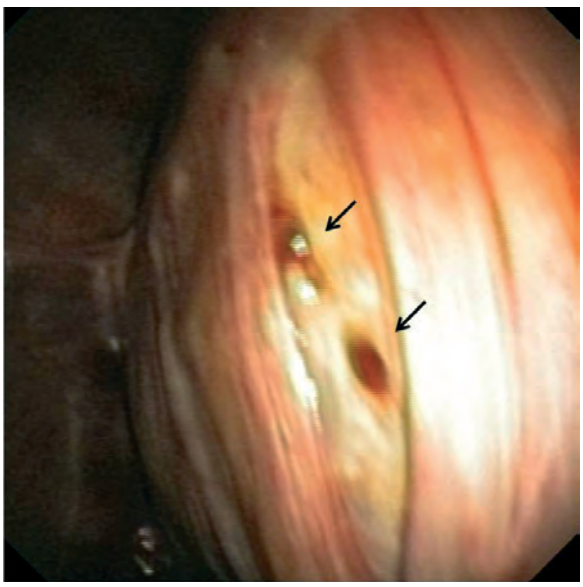


Fig. 1. Medical thoracoscopy revealing flat brownish gray plaques (black arrows) with hematoma over diaphragmatic pleura.

Biopsies were obtained from parietal and diaphragmatic pleura and they revealed fragments of endometrial glands lined by columnar cells surrounded by endometrial stroma along with hemosiderin-laden macrophages (Fig. 2).

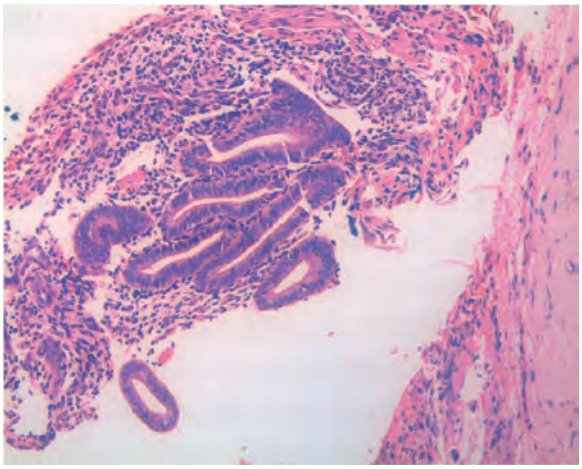


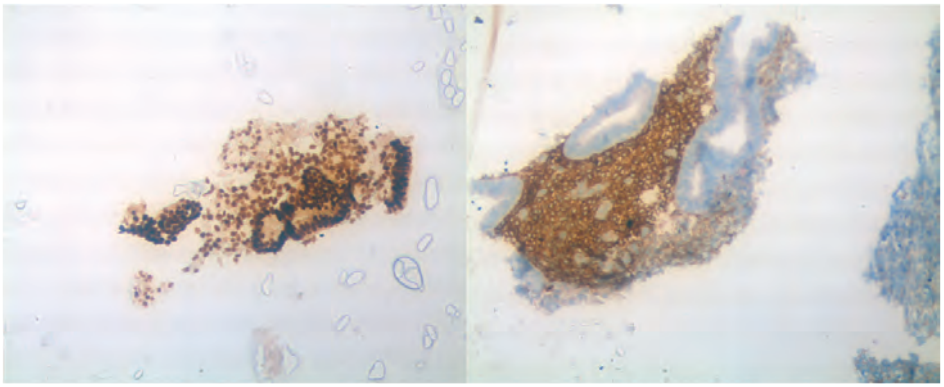
Fig. 2. Microphotograph of the biopsy specimen showing foci of endometrial glands surrounded by endometrial stroma. (H&E ×20).

The pleural tissue stained positive for estrogen receptor and CD 10 immunostains (Fig. 3).

As the patient presented with pleural effusion the possible etiology considered was for:

- Tuberculosis;
- Malignancy;
- Secondary to connective tissue disease;
- Ovarian hyperstimulation syndrome secondary to clomiphene.

Tuberculosis and malignancy were ruled out by histopathology of pleural tissue obtained by medical thoracoscopy. Similarly, ovarian hyperstimulation was ruled out clinically by absence of features like weight gain, abdominal pain, vomiting, ascites and bilateral pleural effusion. Connective tissue disease was ruled out by absence of symptoms, clinical features and serology. Patient was diagnosed to have pleural endometriosis and was started on leuprolide (gonadotropin releasing hormonal analogue) after getting expert opinion from gynecologist. Post second day lung thoracoscopy showed adequate expansion and intercostal drainage was removed. She was discharged on leuprolide with the advice to review after three months. The patient and her family were educated about the nature of disease; treatment options and the need of regular follow up. After three months on follow up the patient showed good clinical and radiological improvement.



Estrogen Receptor Positivity **CD10 positivity**

Fig. 3. Immunohistochemical analysis demonstrating positive staining estrogenic receptor and CD 10 stains (Original magnification ×20×digital magnification).

Discussion

Maurer in 1958 for the first time described thoracic endometriosis; it is associated with growth of endometrial tissue in the bronchial tree, lung parenchyma and pleura [1, 3]. Thoracic endometriosis is a rare uncommon entity and usually it affects right hemi-thorax [3] in 92% and left in 5% of cases. Pleural cavity is involved more commonly than lung parenchyma. The disease usually affects nulliparous women in their child bearing ages with a peak incidence between 20-30 years of age. The common presentations of thoracic endometriosis syndrome (TES) are pneumothorax (70%), hemothorax (12%), hemoptysis (7%) and lung nodules (6%) [4]. Chest pain, hemoptysis and breathing difficulty are common clinical manifestations of TES and symptoms typically present in 48-72 hours after start of menstruation.

The exact etiopathogenesis of TES is unknown, but many hypotheses have been suggested for presence of endometrial tissue in the thoracic cavity. Pleural TES is explained by Ivanoff's metaplasia theory (local metaplasia of cholemic epithelium) and Sampson's retrograde menstruation theory (trans diaphragmatic pass of endometrial tissue and implantation in thoracic cavity). Hematogenous migration of endometrial tissue via vascular and lymphatic micro embolization after gynaecological procedures like caesarean and curettage have been suggested as causes for parenchymal TES (hematogenous migration theory/micro-embolization theory) [1].

TES is diagnosed clinically based on presence of symptoms during menstruation [1, 5]. Histopathological confirmation is needed for definitive diagnosis. Thoracoscopy is increasingly used as the diagnostic modality for obtaining tissue for histology. As in our case, pleural and diaphragmatic implants can be seen in thoracoscopy and to increase the yield it is better to perform the procedure during the menstruation [6]. Medical treatment of TES includes hormonal manipulation and it involves suppression of ectopic endometrium with oral contraceptive pills, danazol, progestins and gonadotropin releasing hormonal analogues [7]. Surgical intervention is reserved of patients in whom the disease recurs after hormonal therapy. Pleurodesis and tissue resection are the treatment modalities surgically performed [7].

Conclusion

Pleural endometriosis and ovarian hyper stimulation syndrome should be among the differentials for pleural effusion especially in females on infertility treatment. Medical thoracoscopy should be considered as the investigation of choice for evaluating pleural effusions before initiating on empirical treatments.

Conflict of Interests

The authors declare no conflict of interest.

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Author's Contributions

Saheer S, Akhil Paul, Prince James – formal analysis, investigation, methodology; *Saheer S, Akhil Paul* – drafting and manuscript revision; *Rayani Palak* – investigation.

ПЛЕВРАЛЬНИЙ ЕНДОМЕТРІОЗ: ГЕМОРАГІЧНИЙ ПЛЕВРАЛЬНИЙ ВИПІТ У 28-РІЧНОЇ ЖІНКИ З ПЕРВИННОЮ БЕЗПЛІДНІСТЮ (КЛІНІЧНИЙ ВИПАДОК)

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Вступ. *Ендометріоз характеризується наявністю ендометрію поза порожниною матки і зазвичай він виявляється в органах малого тазу. Ектрагенітальна його форма – ендометріоз легень - зазвичай маніфестує у вигляді пневмотораксу, кровохаркання, гемотораксу або легеневих вузликів. Вкрай рідко зустрічаються повідомлення про ендометріоз легень з клінічними проявами геморагічного плеврального випоту.*

Мета. *Метою дослідження було описати ендометріоз легень, що маніфестував як геморагічний плевральний випіт та показати важливість медичної торакоскопії у постановці діагнозу.*

Методи. *Описано клінічний випадок рідкісної форми ендометріозу легень - геморагічного плеврального випоту, який не розсмоктувався.*

Результати. *Представлено клінічний випадок 28-річної жінки, яка лікувалася з приводу первинного безпліддя, з геморагічним плевральним випотом, який не розсмоктувався. Через підозру на туберкульоз протягом останніх чотирьох місяців вона приймала призначені лікарем чотири протитуберкульозних препарати. Медична торакоскопія виявила плоскі коричнево-сірі бляшки над діафрагмальною плеврою, а гістологія плевральної тканини виявила ендометріоз плеври. Пацієнтці було призначено лейпроліду ацетат (синтетичний агоніст гонадотропін-рилізинг гормону), після чого спостерігалося клініко-рентгенологічне покращення.*

Висновки. *При проведенні диференціальної діагностики плеврального випоту у пацієнток, особливо при лікуванні безпліддя обов'язково слід розглядати ймовірність ендометріозу легень. Медична торакоскопія повинна бути методом вибору при оцінці плеврального випоту перед початком емпіричного лікування.*

КЛЮЧОВІ СЛОВА: **ендометріоз легень; катаменіальний плевральний випіт; геморагічний плевральний випіт.**

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References

1. Alifano M, Trisolini R, Cancellieri A, Regnard JF. Thoracicendometriosis: current knowledge. Ann Thorac Surg. 2006; 81:761-9.
DOI: https://doi.org/10.1016/j.athoracsur.2005.07.044

2. Sevinç S, Unsal S, Oztürk T et al. Thoracic endometriosis syndrome with bloody pleural effusion in a 28 year old woman. J Pak Med Assoc. 2013 Jan; 63:114-6.

3. Rived X, Sebastián F, Rodríguez JI et al. Endometriosispleural complicada. Neumotórax catamenial. Cir Esp. 2002; 67:114-5.

4. Joseph J, Sahn SA. Thoracic endometriosis syndrome: new observations from an analysis of 110 cases. J Med. 1996;100:164-70.
DOI: https://doi.org/10.1016/S0002-9343(97)89454-5

5. Channabasavaiah AD, Joseph JV. Thoracic endometriosis: revisiting the association between clinical presentation and thoracic pathology based on thoracoscopic findings in 110 patients. Medicine (Baltimore). 2010;89:183- 8.
DOI: https://doi.org/10.1097/MD.0b013e3181df67d5
6. Bagan P, Le Pimpec Barthes F, Assouad J et al. Catamenial pneumothorax: retrospective study of surgical treatment. Ann Thorac Surg. 2003;75:378- 81.
DOI: https://doi.org/10.1016/S0003-4975(02)04320-5
7. Alifano M, Jablonski C, Kadiri H et al. Catamenial and noncatamenial, endometriosis-related or nonendometriosis-related pneumothorax referred for surgery. Am J Respir Crit Care Med. 2007;176:1048- 53.
DOI: https://doi.org/10.1164/rccm.200704-587OC

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DIAGNOSTIC UTILITY OF LEUKOCYTE PARAMETERS IN THE PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Background. *Inflammation is one of the key players in acute myocardial infarction (AMI). One of the ways to evaluate it indirectly is to analyze leukocyte parameters of complete blood count (CBC), which is a routine and affordable method of diagnosis. Leukocyte counts can provide additional information about the course, as well as a potential prognosis for complications of AMI. We suggest that the dynamic changes of CBC during the treatment of the patients with AMI may be of value to assess the prognosis of the course of the disease, and therefore require further study.*

Objective. *The aim of the study to evaluate the diagnostic and prognostic potential of leukocyte indexes of CBC, in particular the levels of leukocytes, lymphocytes, neutrophils and N/L, WBC/MPV, PLT/L ratios in the patients with AMI at the time of hospitalization and on the 7th day of hospital stay.*

Methods. *The study involved 204 individuals: 152 patients with AMI (Group 1), 30 patients with stable coronary heart disease (Group 2) and 24 healthy volunteers (Group 3). Hemogram parameters and their ratios, in particular the levels of leukocytes, lymphocytes, neutrophils, ESR, as well as the ratios of N/L and PLT/L were studied.*

Results. *Levels of leukocytes, neutrophils, lymphocytes, as well as N/L, WBC/MPV, MPV/L ratios were significantly higher in the patients with AMI compared to other groups. The best diagnostic value had such indicators as the total number of leukocytes (sensitivity 71.7%, specificity 69.7%, AUC 0.794), the absolute number of granulocytes (sensitivity 81.7%, specificity 77.4%, AUC 0.803), the N/L ratio (sensitivity 75.0%, specificity 71.7%, AUC 0.791) and the WBC/MPV ratio (sensitivity 76.7%, specificity 62.3%, AUC 0.760). The PLT/L ratio calculated on the 7th day of hospital stay correlated with the risk of in-hospital (r=0.369, p=0.002) and 6-month mortality (r=0.338, p=0.004) according to the GRACE score.*

Conclusions. *Leukocytes, granulocytes, N/L and WBC/MPV ratios had a fairly high diagnostic value for the patients with AMI. Regarding the prognostic potential assessment, only the PLT/L ratio on the 7th day of hospitalization correlated with the risk of in-hospital and 6-month mortality. This proves the importance of assessing CBC parameters not only at the time of hospitalization, but also in the dynamics of AMI.*

KEYWORDS: **inflammation; acute myocardial infarction; complete blood count; leukocytes.**

Introduction

Inflammation plays a key role in the pathophysiology of acute myocardial infarction (AMI), both at the time of its development and later on [1, 2]. Leukocytes are the main contributors in the formation and subsequent destabilization of fibroatheroma. At the same time, they are closely related to activation of platelets increasing their prothrombotic potential [3, 4]. Different white blood cell (WBC) subtypes play their own role in this process. There are several studies that prove not only the diagnostic but also the prognostic value of assessment of leukocyte indexes and their ratios in management of the patients with AMI [5–7]. However, most of these works are focused on the complete blood count (CBC) parameters only at the time of hospitalization. We suggest that the dynamic changes of CBC during treatment of the patients with AMI may be of value to assess the prognosis of the disease course and therefore require further study.

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Methods

The study involved 152 inpatients with AMI – Group 1. Besides, two more study groups were formed: a comparison group, which included patients with stable ischemic heart disease (SIHD) (n=30) – Group 2, and a control group of healthy volunteers (n=28) – Group 3. The study was carried out at Ternopil City Municipal Hospital No. 2 (Ukraine) in October 2018 – June 2019.

The diagnosis of AMI was verified following the clinical guidelines [8, 9]. Clinical symptoms of AMI, in particular the duration of angina pectoris and symptom-to-balloon time, the results of biochemical blood tests, angiographic protocols were evaluated. In addition, the risk of complications for acute coronary syndrome was assessed using the GRACE score [10, 11].

Blood sampling was performed from the ulnar vein in the first hour and on the 7th day after hospitalization. CBC was performed using an automated hematology analyzer; in addition, a manual count of leukocyte fractions in peripheral blood was performed, as well as the erythrocyte sedimentation rate measurement (ESR).

Statistical data was processed using the application package SPSS v25.0, as well as Microsoft Excel spreadsheets. The data were presented in the format of "mean ± standard error of the mean". The significance of the differences between the two independent samples in the case of normal data distribution was determined using the Student's t-test; in the case of abnormal data distribution – the non-parametric Mann-Whitney test. To compare three or more independent samples, one-factor analysis of variances was used – the ANOVA (analysis of variance), in the case of abnormal data distribution – the Kruskal-Wallis ranking criterion.

The ROC analysis was used to identify the diagnostic value of individual ZAK indicators. Such parameters as an area under the curve (AUC), as well as the sensitivity and specificity of the test, were analyzed.

The Pearson's correlation analysis was used to assess the relationships between the studied indicators with a normal distribution of data, and the Spearman's correlation analysis – in the case of abnormal distribution. The null hypothesis was denied at $p<0.05$. The correlation coefficient was evaluated according to the following criteria: $r<0.3$ – weak relationship; $r=0.30–0.49$ – moderate; $r=0.50–0.69$ – significant; $r=0.70$ – 0.89 – strong; $r>0.90$ – very strong, close to the functional association.

Results

A mean age of the patients with AMI was (62.91±10.90) years old, among them the male predominated (n=116, 76.3%). More than half of the subjects had concomitant pathology in the form of hypertension (n=134, 88.16%) and diabetes mellitus (n=29, 19.08%).

Comparing the parameters of the CBC between the three groups (Table 1) it was established that in the group of patients with AMI the level of leukocytes was significantly higher than in the control and comparison groups. There was no significant difference in

Table 1. Comparison of CBC parameters between the patients with AMI, SIHD and healthy volunteers

	Group 1 (n=152)	Group 2 (n=30)	Group 3 (n=24)	P-value (ANOVA)	P value
Age, years	63.11±0.89	56.13±1.66	45.75±3.30	<0.001	0.01[a,b,c]
Men, n (%)	116 (76.3)	24 (77.4)	17 (70.8)	NS	NS
WBC, 10^9/L	9.10±0.24	6.89±0.26	6.45±0.35	<0.001	<0.001[a,b]
Lymphocytes, 10^9/L	1.85±0.13	2.14±0.12	2.40±0.18	0.038	0.023[b]
Monocytes, 10^9/L	0.51±0.06	0.64±0.16	0.78±0.20	NS	NS
Granulocytes, 10^9/L	7.01±0.41	5.29±0.94	3.66±0.33	0.001	<0.001[b]
N/L ratio	5.54±0.64	2.53±0.31	1.64±0.17	<0.001	<0.001[a,b] 0.015[c]
WBC/MPV ratio	1.03±0.05	0.76±0.03	0.78±0.04	<0.001	<0.001[a] 0.002[b]
MPV/L ratio	7.00±0.66	4.63±0.36	5.13±0.31	0.001	0.002[a] <0.001[b]
PLT/L ratio	161.59±11.86	113.99±9.07	114.36±7.83	0.004	0.02[a] 0.001[b]
ESR, mm/hour	11.20±0.75	8.26±0.81	7.71±0.92	0.048	<0.05[a,b]

Notes:
a – comparing groups 1 and 2, b – comparing groups 1 and 3, c – comparing groups 2 and 3.
NS – not significant
WBC – white blood cells, N/L ratio – neutrophils to lymphocytes ratio, WBC/MPV ratio – white blood cells to mean platelet volume ratio, MPV/L ratio – mean platelet volume to lymphocyte ratio, PLT/L ratio – platelet count to lymphocytes ratio, ESR – erythrocyte sedimentation rate.

the absolute number of lymphocytes and granulocytes between the AMI and comparison groups, but such differences were found when comparing the AMI patients and healthy volunteers: in the AMI group, there was a significantly lower lymphocyte count and higher granulocytes. The N/L ratio differed between the three groups and was the highest in the patients with AMI ($p<0.001$).

The ratios that simultaneously reflected two links in the pathogenesis of coronary heart disease: inflammation and platelet activation, such as PLT/L, WBC/MPV and MPV/L, were of particular interest. All of them were significantly higher in the patients with AMI, compared with the control and comparison groups.

Taking into account the obtained results of average values comparison, the diagnostic value was determined, as well as the sensitivity and specificity of certain parameters of the CBC in the patients with AMI (Fig. 1).

As seen in Table 2, the indicators such as total leukocyte count, absolute granulocyte count, N/L ratio, and WBC/MPV ratio were of the best diagnostic value.

A negative correlation was found between symptom-to-balloon time and the absolute level of lymphocytes ($r=-0.38$; $p=0.008$), while the positive correlation was determined with the following indicators: the N/L ($r=0.370$; $p=0.07$), PLT/L ($r=0.380$; $p=0.06$) and MPV/L ($r=0.351$; $p=0.011$) ratios. Also relationships were found between the duration of angina, i.e. the duration of ischemia and the number of granulocytes ($r=0.366$; $p=0.004$), the N/L ($r=0.370$; $p=0.004$) and PLT/L ($r=0.260$; $p=0.045$) ratios and the number of lymphocytes ($r=-0.268$; $p=0.038$).

The relationship between the level of CPK MB and the N/L ratio ($r=0.567$; $p<0.001$), as well as hematocrit ($r=0.406$; $p=0.004$) was also established.

Any significant relationships between CBC and prognostic markers of AMI, such as the GRACE score, have not been established at this stage.

The dynamics of general blood test in the patients with AMI were further analyzed (Table 3).

A decrease in the levels of leukocytes and neutrophils was evidenced ($p<0.001$ and $p=0.012$). Instead, the ESR increased significantly.

Discussion

High levels of neutrophils in the patients with AMI found in our study are associated with

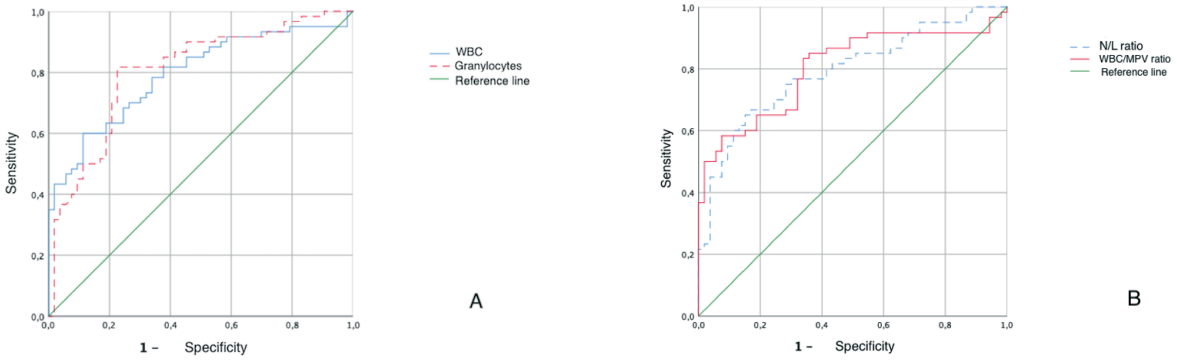

Fig. 1. Receiver operating characteristics analysis of leukocyte parameters in the AMI patients.

Table 2. Diagnostic sensitivity and specificity of the CBC parameters in the AMI patients

	AUC	Sensitivity, %	Specificity, %	Cut-off point
WBC, 10^9/L	0.794	71.7	69.7	7.61
Granulocytes, 10^9/L	0.803	81.7	77.4	4.99
N/L ratio	0.791	75.0	71.7	2.40
Plt/L ratio	0.654	65.0	52.8	108.49
WBC/MPV ratio	0.760	76.7	62.3	0.85
MPV/L ratio	0.051	66.7	64.2	4.44

Notes: AUC – area under the curve. WBC – white blood cells, N/L ratio – neutrophils to lymphocytes ratio, WBC/MPV ratio – white blood cells to mean platelet volume ratio, MPV/L ratio – mean platelet volume to lymphocyte ratio, PLT/L ratio – platelet count to lymphocytes ratio, ESR – erythrocyte sedimentation rate.

Table 3. Comparison of CBC parameters in the AMI patients at the time of hospitalization and on the 7th day after MI

	Day 1	Day 7	P value
WBC, 10^9/L	9.34±0.32	8.07±0.27	<0.001
Lymphocytes, 10^9/L	2.10±0.19	2.09±0.17	NS
Monocytes, 10^9/L	0.57±0.11	0.71±0.17	NS
Granulocytes, 10^9/L	6.74±0.50	5.30±0.49	0.012
N/L ratio	4.28±0.63	2.92±0.29	NS
WBC/MPV ratio	1.06±0.8	0.88±0.05	NS
MPV/L ratio	7.16±1.02	4.58±0.28	0.015
Plt/L ratio	141.45±14.50	145.31±12.05	NS
ESR, mm/hour	12.34±1.21	22.88±1.45	<0.001

Notes. WBC – white blood cells, N/L ratio – neutrophils to lymphocytes ratio, WBC/MPV ratio – white blood cells to mean platelet volume ratio, MPV/L ratio – mean platelet volume to lymphocyte ratio, PLT/L ratio – platelet count to lymphocytes ratio, ESR – erythrocyte sedimentation rate.

the formation of platelet-leukocyte aggregates in the lumen of the vessel that leads to their increase in the area of myocardial infarction. Also, neutrophils can affect platelet function by direct adhesion or by secretory factors. In contrast, the number of lymphocytes in AMI tends to decrease. We were able to confirm this pattern when comparing patients with AMI and healthy volunteers, at the same time there were no differences in the group of patients with stable coronary heart disease. This can be explained by the fact that the decrease in the number of lymphocytes is associated with physiological stress, which leads to increased cortisol levels and activates the process of apoptosis in the lymphocytes [12]. Thus, in the case of early admission to the hospital, this index may simply not have time to decrease. This is confirmed by the correlation we found, according to which the longer the angina pectoris lasted, the lower the patient's lymphocyte level was.

Given that neutrophils and lymphocytes are the cells with the opposite effect in the context of vascular inflammation, it is important to assess not only their absolute numbers but also the balance between them. One of the potential ways to do this is to evaluate the N/L ratio, which is considered to be an indicator of systemic inflammation [13]. According to our results, it not only significantly differed in the patients from the study and comparison groups, but also proved a significant positive correlation with the marker of myocardial necrosis – CPK MB.

When assessing the hemogram on the 7th day, a significant decrease in the level of leukocytes, due to neutrophils, and an increase in ESR were noted. This phenomenon is also known as the "scissors symptom" or "crossover symptom" and is an evidence of the necro-resorptive syndrome, denoted by a systemic inflammatory response to the entry of myocardial breakdown products into the bloodstream. Thus, the number of neutrophils, which is traditionally the highest on the 1st-3rd day after the development of AMI, dets to norm on the 5th-7th days due to active phagocytosis of dead neutrophils in the infarct area provided by macrophages [10]. The increase of ESR is caused by the fact that normally the charge of the erythrocyte membrane is negative and this allows them to repel each other. At the same time, pro-inflammatory proteins, in particular fibrinogen and C-reactive protein, have a positive charge and can significantly affect the state of the erythrocyte membrane causing their further aggregation [11].

When assessing the prognostic potential of individual indicators of CBC, we were able to identify it only in relation to the PLT/L ratio on the 7th day of AMI. The fact that the PLT/L ratio correlated with the risk of mortality only in 1 week is most likely explained by the platelet resistance to antiplatelet therapy during the treatment course, as well as to the stimulating effect of inflammation on megakaryocyte proliferation [14, 15]. The advantage of PLT/L ratio, compared to individual leukocyte and platelet indices, is that it reflects two interdependent processes of inflammation and platelet activation and has already proven to be a good prognostic marker of AMI, in particular regarding the prediction of left ventricular (LV) thrombus, remodeling of LV in the post-infarction period, as well as all-case mortality [14, 16–18].

Conclusions

Levels of leukocytes, granulocytes, lymphocytes, as well as N/L, WBC/MPV ratios, are available and informative markers that provide additional information about the processes of inflammation during the acute phase of myocardial infarction.

We were unable to establish a link between CBC indexes at the time of hospitalization and prognostic factors of AMI. Instead, the parameters that were obtained on the 7th day of treatment, in particular the PLT/L ratio was associated with the risk of in-hospital and 6-month mortality. Thus, we emphasize the importance of assessing the CBC indexes and ratios in the patients with AMI in the dynamics as an inexpensive and informative method for Post-MI risk stratification.

Conflicts of Interest

Authors declare no conflict of interest.

Author's Contributions

Diana V. Zhehestovska **–** investigation, conceptualization, data curation, formal analysis, writing – original draft.

Marian V. Hrebenyk **–** data curation, writing – reviewing and editing.

ДІАГНОСТИЧНІ МОЖЛИВОСТІ ЛЕЙКОЦИТАРНИХ ПОКАЗНИКІВ СЕРЕД ПАЦІЄНТІВ З ГОСТРИМ ІНФАРКТОМ МІОКАРДА

Д.В. Жегестовська, М.В. Гребеник

ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Запалення відіграє визначальну роль в процесі розвитку та перебігу гострого інфаркту міокарда (ГІМ). Аналіз лейкоцитарних показників загального аналізу крові (ЗАК) є одним із способів його опосередкованої оцінки, це рутинний і доступний метод діагностики. Ми припускаємо, що динамічні зміни параметрів ЗАК в процесі лікування пацієнтів з ГІМ можуть становити цінність, зокрема і для оцінки прогнозу подальшого перебігу захворювання, а тому потребують подальшого вивчення.*

Мета*. Оцінити діагностичний та прогностичний потенціал лейкоцитарних індексів ЗАК, зокрема кількісних рівнів лейкоцитів (WBC), лімфоцитів (L), нейтрофілів (N) та співвідношень N/L, WBC/MPV (середнього рівня тпромбоцитів, MPV), PLT/L (співвідношення числа тромбоцитів до кількості лімфоцитів) у пацієнтів з ГІМ на момент госпіталізації та на 7-му добу перебування в стаціонарі.*

Матеріали та методи*. В дослідженні взяло участь 204 особи (152 пацієнтів з ГІМ (Група 1), 30 пацієнтів з стабільною ІХС (Група 2) та 24 здорові добровольці (Група 3)). Досліджували показники гемограми та їх співвідношення, зокрема рівні лейкоцитів, лімфоцитів, нейтрофілів, ШОЕ, а також співвідношень N/L ratio та PLT/L ratio.*

Результати. *Рівні лейкоцитів, нейтрофілів, лімфоцитів, а також відношення N/L, WBC/MPV, MPV/L були достовірно вищими серед пацієнтів з ГІМ порівняно із іншими групами. Найкращою діагностичною цінністю володіли такі показники, як загальна кількість лейкоцитів (чутливість 71,7%, специфічність 69,7%, AUC 0,794), абсолютна кількість гранулоцитів (чутливість 81,7%, специфічність77,4%, AUC 0,803), відношення N/L (чутливість 75,0%, специфічність 71,7%, AUC 0,791) та WBC/MPV (чутливість 76,7%, специфічність 62,3%, AUC 0,760). Співвідношення PLT/L вирахуване на 7-ий день перебування в стаціонарі корелювало з ризиком госпітальної (r = 0,369, p = 0,002) та 6-ти місячної смертності (r = 0,338; p = 0,004).*

Висновки. *Показники лейкоцитів, гранулоцитів та співвідношення N/L і WBC/MPV володіли доволі високою діагностичною цінністю серед пацієнтів з ГІМ. Стосовно оцінки прогностичного потенціалу, то лише відношення PLT/L на 7-ий день госпіталізації корелювало з ризиком госпітальної та 6-ти місячної смертності. Це вказує на важливість оцінки ЗАК не лише на момент госпіталізацій, а й в динаміці перебігу ГІМ.*

КЛЮЧОВІ СЛОВА: **запалення; гострий інфаркт міокарда; загальний аналіз крові; лейкоцити.**

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References

1. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res. 2002;53:31–47.

2. Ferrari JP, Lueneberg ME, da Silva RL, Fattah T, Gottschall CAM, Moreira DM. Correlation between leukocyte count and infarct size in ST segment elevation myocardial infarction. Arch Med Sci Atheroscler Dis. 2016;1:e44–e48.

3. Italiano JE, Hartwig JH. Chapter 2 – Megakaryocyte Development and Platelet Formation. In: Michelson ADBT-P (Third E (ed). Academic Press; 2013. p. 27-49.

4. Korniluk A, Koper-Lenkiewicz OM, Kamińska J, Kemona H, Dymicka-Piekarska V. Mean platelet volume (MPV): new perspectives for an old marker in the course and prognosis of inflammatory conditions. Mediators of inflammation. 2019 Oct;2019.

DOI: https://doi.org/10.1155/2019/9213074

5. Lassale C, Curtis A, Abete I, van der Schouw YT, Verschuren WMM, Lu Y, Bueno-de-Mesquita HB. Elements of the complete blood count associated with cardiovascular disease incidence: Findings from the EPIC-NL cohort study. Sci Rep. 2018;8:3290.

6. Li J, Wu J, Zhang M, Zheng Y. Dynamic changes of innate lymphoid cells in acute ST-segment elevation myocardial infarction and its association with clinical outcomes. Sci Rep. 2020;10:5099.

7. Khode V, Sindhur J, Kanbur D, Ruikar K, Nallulwar S. Mean platelet volume and other platelet volume indices in patients with stable coronary artery disease and acute myocardial infarction: A case control study. J Cardiovasc Dis Res. 2012;3:272–75.

8. Collet J-P, Thiele H, Barbato E, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J. 2020.

DOI: https://doi.org/10.1093/eurheartj/ehaa575

9. Ibanez B, James S, Agewall S, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J. 2018;39:119–77.

10. Granger CB, Goldberg RJ, Dabbous O, et al. Predictors of Hospital Mortality in the Global Registry of Acute Coronary Events. Arch Intern Med. 2003;163:2345–53.

11. Eagle KA, Lim MJ, Dabbous OH, et al. A Validated Prediction Model for All Forms of Acute Coronary SyndromeEstimating the Risk of 6-Month Postdischarge Death in an International Registry. JAMA. 2004;291:2727–33.

12. Hofmann U, Frantz S. Role of lymphocytes in myocardial injury, healing, and remodeling after myocardial infarction. Circ Res. 2015;116:354–67.

13. Kalay N, Dogdu O, Koc F, Yarlioglues M, Ardic I, Akpek M, Cicek D, Oguzhan A, Ergin A, Kaya MG. Hematologic parameters and angiographic progression of coronary atherosclerosis. Angiology. 2012;63:213–17.

14. Azab B, Shah N, Akerman M, McGinn JT. Value of platelet/lymphocyte ratio as a predictor of all-cause mortality after non-ST-elevation myocardial infarction. J Thromb Thrombolysis. 2012;34:326–34.

15. Alexandrakis MG, Passam FH, Moschandrea IA, Christophoridou A V, Pappa CA, Coulocheri SA, Kyriakou DS. Levels of serum cytokines and acute phase proteins in patients with essential and cancer-related thrombocytosis. Am J Clin Oncol. 2003;26:135–40.

16. Zhang Q, Si D, Zhang Z, Wang C, Zheng H, Li S, Huang S, Zhang W. Value of the platelet-to-lymphocyte ratio in the prediction of left ventricular thrombus in anterior ST-elevation myocardial infarction with left ventricular dysfunction. BMC Cardiovasc Disord. 2020;20:1–8

17. Bekler A, Gazi E, Yılmaz M, Temiz A, Altun B, Barutçu A, Peker T. Could elevated platelet-lymphocyte ratio predict left ventricular systolic dysfunction in patients with non-ST elevated acute coronary syndrome? Anatol J Cardiol. 2015;15:385–90.

18. Akdag S, Akyol A, Asker M, Ozturk F, Gumrukcuoglu HA. The relation of platelet-lymphocyte ratio and coronary collateral circulation in patients with non-ST segment elevation myocardial infarction. Postep w Kardiol Interwencyjnej. 2016;12:224–30.

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EFFECTS OF HEMOTOXIC SNAKE BITE ENVENOMATION ON HAEMATOLOGICAL PARAMETERS VARIABILITY IN PREDICTING COMPLICATIONS

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Background. *Snake bite envenomation is a major public health problem in India with a high mortality rate. The major complications following a hemotoxic snake bite are disseminated intravascular coagulation (DIC), shock, acute kidney injury (AKI), acute respiratory distress syndrome (ARDS) and coagulopathy. The study explores a possible correlation of the haematological parameters studied to complications like DIC, AKI, acute renal failure (ARF), ARDS, shock and gastrointestinal (GI) bleed.*

Objective. *The aim of the study was to find out the effect of snakebite envenomation on the major haematological parameters of the body in relation to complications.*

Methods. *This cross-sectional study was conducted during a period of 18 months. 150 patients were included in the study after obtaining their informed consents. Data collection was done using a proforma. The study also compared clotting time (CT) by capillary tube method and whole blood clotting time at 20 minutes (WBCT20). SPSS software was used for statistical analysis.*

Results. *Among the people with complications, the majority (52%) of participants had AKI, 26% of them requiring dialysis, 16.7% participants had GI bleed, 11.3% participants had shock and 10% participants had DIC.*

Conclusions. *A prolonged bleeding time was found to be one of the most helpful haematological parameters in predicting shock and AKI. Clotting time by both capillary tube and WBCT20 methods were comparable in predicting complications.*

KEYWORDS: **hemotoxic snake bite; acute kidney injury; shock; clotting time; bleeding time.**

Introduction

Snakebite envenomation is a major public health problem in India with a high mortality rate [1]. The WHO estimates that there are 81000 snake envenomation in India per annum with a mortality of 11000 (13.5%) [2]. It has been reported that there are 5 million snake bites with 2.5 million envenomation and 125,000 fatalities worldwide annually [3]. India is reported to have the highest number of snake bites (81,000) and deaths (11,000) per year [3]. Snakebite is considered as one of the neglected tropical diseases by the WHO [4]. The mortality due to snakebites in India is due to various socioeconomic, cultural and environmental causes [5]. Among the 52 species of venomous snakes in India, 4 species are responsible for greatest number of snake bites which are medically significant. These are referred to as the big four, *Bungarus caeruleus* (common krait), *Daboia russelii* (Russel's viper), *Echis carinatus* (saw scaled viper), *Naja naja* (Indian cobra). But recently it has been discovered that another species, the hump-pit viper (*Hypnale hypnale*), is capable of causing lethal envenomation [6].

The venomous snakes found in India can be classified per the type and action of venom as Neurotoxic (Cobra, Krait) and Hemotoxic (Vipers). Then main complaints are pain and swelling at the site of snake bite [7–11].

The major complications following hemotoxic snake bite are disseminated intravascular coagulation (DIC), shock, acute kidney injury (AKI), acute respiratory distress syndrome (ARDS) and coagulopathy [12]. AKI is one of the serious complications developing after snake bite [13,14]. Early identification of hemotoxic envenomation and anti-snake venom (ASV) administration may help decrease morbidity and mortality [15].

The study tries to find out the common complications after hemotoxic snake bites and to explore a possible correlation of the haematological parameters to complications. The study also compares clotting time by capillary tube method and WBCT20. ASV administration is currently based on prolongation of WBCT20. Capillary tube method for estimating CT could be used in a resource poor setting, if the

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predictability of the test is comparable to that of WBCT20. In a study by Ratnayake et al [16] the diagnostic utility of WBCT20 was determined at the time of admission and it was found out that it had got sensitivity of 82% and specificity of 98% in detecting venom induced consumption coagulopathy (VICC). The aim of the study was to assess the value of haematological parameters, i.e. bleeding time (BT), clotting time (CT), prothrombin time (PT), activated partial thromboplastin time (aPTT) in predicting the complications. The primary objective was to assess the effect of snake bite on the major haematological parameters of the body in relation to complications.

Methods

This cross-sectional study was conducted over a period of 18 months after obtaining clearance from the institutional ethics committee. A total of 150 patients, who were above 18 years of age, had given written informed consent and had clinical features of hemotoxic envenomation or increase of any of the evaluated haematological parameters as bleeding time (BT), clotting time (CT), prothrombin time (PT), activated partial thromboplastin time (aPTT), were included in the study. Convenient sampling was done.

The patients who had not given written informed consent and the patients with bites other than hemotoxic snakebites were excluded from the study. Any patient who is a known case of bleeding disorder, chronic alcoholic, with acute or chronic liver disease, pregnant females and on anticoagulation therapy were excluded from the current study. After admission a complete history was taken and a thorough clinical examination was conducted. As per the protocol followed in our Institute, all patients bitten by poisonous snakes received polyvalent anti snake venom (ASV), within 15 minutes of reaching the hospital, provided they had clinical features of envenomation. The patients with no initial features of envenomation were administered with ASV as soon as they exhibited first signs of envenomation.

Initial blood samples collected from the patients admitted with hemotoxic envenomation were sent for complete hemogram, random blood sugar (RBS), renal function tests (RFT), liver function tests (LFT), serum electrolytes and important coagulation parameters including BT, CT (both WBCT20 and capillary tube methods), PT with INR, D-dimer and a PTT. All patients with features of envenomation were administered with 10 vials of ASV. The response was monitored clinically by doing WBCT20 and Clotting Time (CT) using the capillary tube method, with 2 hour intervals if prolonged initially, otherwise with 6 hour intervals.

If the features of envenomation were persistent or if the WBCT20 was prolonged after 6 hours of first dose, a repeated dose of 10 vials of ASV was given. Blood parameters like BT, CT, PT, APTT and D-dimer were investigated as per the protocol. The first sample of blood was taken on admission for evaluating BT, CT, PT with INR, and APTT. BT was subsequently estimated 2 times with a 5 minute interval, then in 2 hours, 4 hours, and 6 hours after admission. Then every 6 hours if prolonged. Once normal, it was repeated once daily. CT was estimated at time of admission. If in norm, CT was repeated with 6 hour intervals for 1 day to test for requirement of ASV. If prolonged, it was tested with 2 hour intervals till the normal rate. CT was estimated by 20-minute whole blood clotting method (WBCT20) and capillary tube method for comparison study. APTT was done on the 1st day and 7th day, but PT was done at the time of admission and daily. D-dimer was done when there was prolongation of CT on 3 consecutive occasions. ASV was given according to the standard protocol with administration of ASV every 6 hours till normal coagulation profile.

A bite was considered hemotoxic, if the culprit snake is identified as hemotoxic or by coagulopathy as suggested by haematological parameters. AKI was defined as the increase of serum creatinine ≥0.3 mg/dL within 48 hours or increase in serum creatininein ≥1.5 times from baseline. AKI staging was performed according to the "Kidney Disease: improving global outcomes" (KDIGO) clinical practice guidelines [17]. Shock was defined by a systolic blood pressure of <90 mmHg with evidence of tissue hypoperfusion (<0.5 mL/kg/h decrease in urine output, cold skin, and or requiring inotropic drugs). The data was collected, occurrence of complications and derangement in haematological parameters were studied and analysed. The data collected was recorded in a standard data collection sheet as per study proforma and later transferred to Microsoft Excel spread sheet for statistical analysis. Complications like AKI, shock were considered as primary outcome variables. CT by capillary tube and WBCT20 at different time periods, BT, PT, aPTT etc. were considered as explanatory variables.

Descriptive analysis was carried out by frequency and proportion for categorical variables. Categorical outcomes were compared between the study groups using the Chi square test / the Fisher's Exact test (if the overall sample size was <20 or if the expected number in any one of the cells was <5, Fisher's exact test was used). P value <0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis [18].

Results

In the study it was observed that the snake bites were more common in males. Out of 150 subjects in our study, 24 (16.0%) cases, were bites by Russel's viper, 4 (2.7%) cases by krait, 2 (1.3%) cases by hump nosed pit viper and 120 (80.0%) were unidentified. The patients included in the study, who had any comorbidity, accounted for 36.0% of the study population (Table 1).

Among the complications, the majority (52%) of participants had AKI, 26% of them required dialysis; 16.7% participants had GI bleed; 11.3% participants had shock and 10% participants had DIC (Table 2).

Association between AKI and shock with various parameters have been shown in the Table below (Table 3). At the time of admission, 11 patients (47.8%) presenting with AKI reported prolonged CT (capillary tube). However, in 6 hours 22 (100%) participants with prolonged CT had AKI. A majority of 36 (60%) participants reported AKI with normal WBCT20 at the time of admission. The occurrence of AKI was evidenced in 30 (81%) participants with prolonged WBCT20 in 6 hours. The occurrence of AKI was found in 17 (58.6%) participants with PT >16.50 seconds. All participants with APTT >35 reported AKI (Table 3). WBCT20 and CT by capillary tube method in 6 hours were comparable in predicting AKI.

Table 1. Summary of baseline parameters in study population (n= 150)

Parameter	Summary (number, %)
Age	
18-30	37 (24.7%)
31-40	30 (20%)
41-50	29 (19.3%)
51-60	27 (18%)
61-65	27 (18%)
Gender	
Male	98 (65.3%)
Female	52 (34.7%)
Place of Bite	
Indoor	1 (0.7%)
Outdoor	149 (99.3%)
Snake Type	
Russel's Viper	24 (16%)
Krait	4 (2.7%)
Hump nosed pit viper	2 (1.3%)
Unidentified	120 (80%)
Classical Bite	43(28.7%)
Local Reaction	65 (43.3%)
Cellulitis	27 (18%)
Gangrene	8 (5.3%)
Ecchymoses/Bleeding	14 (9.3%)
Comorbidities	54 (36%)
Pain at bite site	128 (83.3%)
Numbness	125 (83.3%)
Sweating	98 (65.3%)
Periorbital oedema	71 (47.3%)
Vomiting	64 (42.7%)
Facial puffiness	48 (32%)

Parameter	Summary (number, %)
Abdominal pain	45 (30%)
Neurological signs	28 (18.7%)
Respiratory distress	25 (16.7%)
Low back ache	20 (13.3%)
Bleeding from any site	15 (10%)
Respiratory failure	15 (10%)
ARDS	11 (7.3%)
Hematemesis	4 (2.7%)
Comorbidities	
Diabetes Mellitus	25 (16.7%)
Hypertension	24 (16%)
Ischemic Heart Disease	0 (0%)
Respiratory Failure	0 (0%)
Bronchial Asthma	11 (7.3%)
Chronic Obstructive Lung Disease	23 (15.3%)
CT prolonged	
At the Time of admission	23 (15.3%)
2 Hours	36 (24%)
4 Hours	29 (19.3%)
6 Hours–12 hours	49 (32.03%)
Day 2	4 (2.7%)
WBC T20-Prolonged	
At the time of admission	90 (60%)
2 Hours	61 (40.7%)
4 Hours	48 (32%)
6 Hours–12 hours	86 (57.3%)
Day 2	15 (10%)

Table 2. Summary of the complications observed (N=150)

Complications	Summary (number, %)
AKI	78 (52%)
Acute Renal Failure (AKI requiring dialysis)	39 (26%)
Dialysis done	39 (26%)
GI Bleed	25 (16.7%)
Shock	17 (11.3%)
DIC	15 (10%)
ARDS	11 (7.3%)
Reaction to ASV	6 (4%)
Mortality	2 (1.3%)
Intra Cerebral Bleed	0 (0%)
Hypopituitarism	0 (0%)
Guillain Barre Syndrome	0 (0%)
Peripheral Neuropathy	0 (0%)

Table 3. Association of different parameters with AKI

Parameter	AKI		P Value
	Present	Absent	
CT capillary tube at the time of admission			
Prolonged (N=23)	11(47.8%)	12 (52.2%)	0.663
Normal (N=127)	67 (52.8%)	60 (47.2%)	
CT capillary tube in 6 hours			
Prolonged (N=22)	22 (100%)	0 (0%)	*
Normal (N=120)	48 (40%)	72 (60%)	
WBCT20 at admission			
Prolonged (N=90)	42 (46.7%)	48 (53.3%)	0.109
Normal (N=60)	36 (60%)	24 (40%)	
WBCT20 at 6 hours			
Prolonged (N=37)	30 (81%)	7 (19%)	<0.001
Normal (N=105)	40 (38%)	65 (62%)	
BT			
>7 (N=12)	12 (100%)	0 (0%)	*
≤7 (N=138)	66 (47.8%)	72 (52.2%)	
PT			
>16.50 (N=29)	17 (58.6%)	12 (41.4%)	0.319
≤16.50 (N=116)	56 (48.3%)	60 (51.7%)	
APTT			
>35 (N=23)	23 (100%)	0 (0%)	*
≤35 (N=123)	51 (41.5%)	72 (58.5%)	

**No statistical tests were applied due to 0 values in one of the cells.*

The occurrence of shock was found in 6 (26.1%) participants with prolonged CT at the time of admission (P value of 0.039). However, 11 (50%) participants had shock with prolonged CT in 6 hours (P value <0.001). The occurrence of shock was found in 11 (12.2%) participants with normal WBCT20 at admission. 17 (42%) participants with prolonged WBC T20 in 6 hours reported shock. The occurrence of shock was found to be statistically significant with p value of <0.001 and the proportion of shock was 8 (66.7%) with BT >7 seconds. The occurrence of shock was found to be significant with a P value of <0.001 in 7 (30.4%) participants with >35 APTT (Table 4).

Table 4. Association of different parameters with shock

Parameter	Shock		P Value
	Present	Absent	
CT capillary tube at the time of admission			
Prolonged (N=23)	6 (26.1%)	17 (73.9%)	0.039
Normal (N=127)	11 (8.7%)	116 (91.3%)	
CT capillary tube at 6 Hours			
Prolonged (N=22)	11 (50%)	11 (50%)	<0.001
Normal (N=120)	6 (5%)	114 (95%)	
WBCT20 at Admission			
Prolonged (N=90)	11 (12.2%)	79 (87.8%)	0.875
Normal (N=60)	6 (10%)	54 (90%)	
WBCT20 at 6 Hours			
Prolonged (N=37)	17 (46%)	20 (54%)	*
Normal (N=105)	0 (0%)	105 (100%)	
BT			
>7 (N=12)	8 (66.7%)	4 (33.3%)	<0.001
≤7 (N=138)	9 (6.5%)	129 (93.5%)	
PT			
>16.50 (N=29)	12 (41.4%)	17 (58.6%)	*
≤16.50 (N=116)	0 (0%)	116 (100%)	
APTT			
>35 (N=23)	7 (30.4%)	16 (69.6%)	<0.001
≤35 (N=123)	6 (4.9%)	117 (95.1%)	

**No statistical tests were applied due to 0 values in one of the cells.*

Discussion

In this study, shock was seen in 11.3% of the total cases, AKI in 52 %, ARF (AKI requiring dialysis) in 26% and gastro intestinal bleed in 16.7%. A comparatively higher incidence of shock was reported by Harshavardhan et al. [19] in their study on hemotoxic snake bite cases (36%). But a comparable value (10.38%) to our study was reported by Kumar et al. [20].

Acute kidney injury (AKI) was established in 52% of the cases in the present study. A somewhat similar value was observed (30.71%) in a previous study [21]. Bhalla et al studied the clinical profile of snake bite in a tertiary centre and observed AKI in 47.6% of the cases [22]. Similar study on outcomes of snakebite poisoning in a tertiary centre by Meenakshi B et al. reported AKI in 44% of cases.

Proteins are an important constituent of snake venoms and they are encoded by polyadenylated mRNA in the venomous glands (12S and 20S) [21]. Besides proteins, the other components present in snake venom include lipids, polysaccharides, nucleotides, nucleosides, free amino acids, riboflavin, serotonin and histamine. Pharmacologically active substances of the venom are enzymes and low molecular weight peptides. The main sites of action of these enzymes are cell membranes, the vascular wall and the blood coagulation cascade [20, 22]. Snake venom, mainly those of Viperidae, contain also molecules that act on the four interconnected blood systems (i) the coagulation system, (ii) fibrinolytic system, (iii) complement and (iv) the kinin system [22].

Shock is consistently the single most important factor in mortality due to Indian viper bite poisoning. The pattern of shock observed shows early transient shock, early sustained shock and late onset shock. Shock is caused by reduction in the circulating blood volume due to a venom-mediated generalized increase in endothelial permeability. Apart from this, massive limb oedema can lead to considerable compartmentalization of blood and plasma. Pulmonary intravascular coagulation, pulmonary oedema caused by increased pulmonary vascular permeability, and cardiotoxic effects of the venom may also be contributory.

The kidney is a highly vascularized organ with excretory function, so it is prone to venom toxicity. AKI, the most significant of all the renal

manifestations, has been reported to be of varying frequency in different studies [23]. Many studies have appeared in literature on snake bite-induced AKI over a decade. Phospholipase A2 is the most widespread and extensively studied of all venom enzymes. It damages mitochondria, red blood cells, leucocytes, platelets, skeletal muscle, vascular endothelium, and other membranes and leads to auto pharmacological release of histamine and anti-coagulants. Viper bites cause various systemic symptoms such as coagulopathy, haemolysis, AKI, a generalized increase in capillary permeability and rhabdomyolysis [23]. Haemoglobinuria caused by intra-vascular haemolysis and myoglobinuria resulting from rhabdomyolysis contributes to development of AKI. Haemorrhage, hypotension, disseminated intravascular coagulation, intravascular haemolysis, and rhabdomyolysis enhance renal ischemia leading to renal failure. Enzymatic activities of snake venoms account for direct nephrotoxicity and immunologic mechanism is insignificant [24].

A variety of factors contribute to shock like fright, hypovolemia (due to extravasation of fluids and blood loss), myocardial depression, haemorrhage into the adrenals and pituitary.

Conclusions

A prolonged clotting time as obtained by capillary tube method in 6 hours is helpful in predicting development of shock. However, since AKI occurred even in those patients having normal clotting time and WBCT20, these parameters may not be useful as predictors of AKI. Prolonged CT by capillary tube method and WBCT20 in 6 hours were comparable in predicting AKI. Prolongation of more than 16.5 seconds in prothrombin time was helpful in predicting the development of shock. However prolonged prothrombin time (>16.50) was not useful in predicting AKI. A prolonged APTT more than 35 seconds was helpful in predicting the onset of shock and AKI. A prolonged bleeding time was found to be one of the most helpful haematological parameters in predicting shock and AKI.

Limitations of the study

A comparatively low number of 150 cases may have strained the generalizations derived by the study. Parameters like CT by capillary tube method did not have a control test measured in patients with non-poisonous snake bite envenomation. A degree of individual variability was inevitable in the evaluation of CT both by capillary tube and WBCT20 methods as well as that of BT. The duration of envenomation prior to admission has not been taken into account in this study, possibly affecting the correlation between haematological parameters and development of complications.

Conflicts of Interest

Authors declare no conflict of interest.

Authors' Contributions

Harikrishnan M.P. – data curation; investigation; methodology**;** supervision; writing – original draft; writing – review & editing.

Anil Kumar C.R. – data curation; investigation; supervision; project administration; methodology**;** supervision; writing – original draft; writing – review & editing.

Anand M.K. – data curation; investigation; methodology**;** supervision; writing – original draft; writing – review & editing.

Jerry Earali – data curation; investigation; methodology**;** supervision; writing – original draft; writing – review & editing.

ВАРІАБЕЛЬНІСТЬ ГЕМАТОЛОГІЧНИХ ПАРАМЕТРІВ ЯК ПРЕДИКТОР РОЗВИТКУ УСКЛАДНЕНЬ ПІСЛЯ ПОТРАПЛЯННЯ В ОРГАНІЗМ ГЕМОТОКСИЧНОЇ ОТРУТИ ВНАСЛІДОК УКУСУ ЗМІЇ

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Вступ. *Одна з найбільших проблем закладів охорони здоров'я в Індії – укуси змій, які супроводжуються високою летальністю. Найбільш часті ускладнення, які розвиваються внаслідок потрапляння в організм гемотоксичної зміїної отрути, це синдром дисемінованого внутрішньосудинного згортання (ДВЗ), шок, гостре ураження нирок (ГУН), гострий респіраторний дистрес синдром (ГРДС) та кооагулопатія.*

Мета дослідження – дослідити можливий взаємозв'язок між частотою розвитку ускладнень як ДВЗ, ГУН, гостра ниркова недостатність (ГНН), ГРДС, шок та кишково-шлункові кровотечі.

Методи. *Це перехресне дослідження проводилося впродовж 18 місяців. Було залучено 150 пацієнтів, які підписали поінформовану згоду. Збір даних проводили за допомогою спеціально розробленої форми. Також досліджували швидкість згортання крові за допомогою капіляра та 20-хвилинного тесту на згортання цільної крові (англ. 20WBCT, показники коагулограми. Для статистичної обробки отриманих даних використовували програмне забезпечення IBM SPSS Statistics.*

Результати. *Серед покусаних людей, у яких розвинулися ускладнення, у більшості (52%) розвинулося ГУН, внаслідок чого 26% серед них потребували діалізу, у 16,7% учасників дослідження були кишково-шлункові кровотечі, у 11,3% - розвинувся шок, у 10% розвинувся ДВЗ.*

Висновки. *Серед досліджуваних гематологічних параметрів для прогнозування розвитку ускладнень (гострого ураження нирок та шоку) після укусу змії найбільш інформативними був подовжений час згортання крові. Обидва методи дослідження згортання крові і за допомогою капіляра і WBCT20 були релевантні та корелювали при прогнозуванні ускладнень.*

КЛЮЧОВІ СЛОВА: **гемотоксична зміїна отрута; укус змії; гостре ураження нирок; шок; час згортання крові; час кровотечі.**

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References

1. Dandona R, Kumar GA, Kharyal A, George S, Akbar M, Dandona L. Mortality due to snakebite and other venomous animals in the Indian state of Bihar: Findings from a representative mortality study. PLoS One 2018;13:e0198900.

DOI: https://doi.org/10.1371/journal.pone.0198900.

2. Kasturiratne A, Wickremasinghe AR, De Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, et al. The global burden of snakebite: A literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Med 2008;5:1591–604.

DOI: https://doi.org/10.1371/journal.pmed.0050218.

3. Menon JC, Joseph JK. Complications of hemotoxic snakebite in India. Toxinology Clin Toxinology Asia Pacific Africa 2015;35:209–32.

DOI: https://doi.org/10.1007/978-94-007-6386-9_22.

4. Kasturiratne A, Wickremasinghe AR, de Silva NG, NK, Pathmeswaran A, Premaratna R, Savioli L, Lalloo DG D, HJ. S. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Med 2008;5:e218.

DOI: https://doi.org/10.1371.

5.Menon J, Joseph JK, Kulkarni K. Treatment of snakebites – a resumé. Cobra 2007;1:1–21.

6. Chippaux JP. Snake-bites: Appraisal of the global situation. Bull World Health Organ 1998;76:515–24.

7. Lavonas EJ, Gerardo CJ, O'Malley G, Arnold TC, Bush SP, Banner W, et al. Initial Experience with Crotalidae Polyvalent Immune Fab (Ovine) Antivenom in the Treatment of Copperhead Snakebite. Ann Emerg Med 2004;43:200–6.

DOI: https://doi.org/10.1016/j.annemergmed.2003.08.009.

8. Yin S, Kokko J, Lavonas E, Mlynarchek S, Bogdan G, Schaeffer T. Factors associated with difficulty achieving initial control with Crotalidae polyvalent immune fab antivenom in snakebite patients. Acad Emerg Med 2011;18:46–52.

DOI: https://doi.org/10.1111/j.1553-2712.2010.00958.x.

9. Gerardo CJ, Evans CS, Kuchibhatla M, Drake WG, Mando-Vandrick JD, Yen M, et al. Time to Antivenom

Administration in Snakebite. Ann Emerg Med 2013; 62:S44.
DOI: https://doi.org/10.1016/j.annemergmed.2013.07.403.
10. Lavonas EJ, Kerns WP, Gerardo CJ, Richardson W, Whitlow K, Berkoff DJ. 328: Long-Term Limb Function Outcomes Following Copperhead Snakebite. Ann Emerg Med 2008;52:S141–2.
DOI: https://doi.org/10.1016/j.annemergmed.2008.06.354.
11. Thorson A, Lavonas EJ, Rouse AM, Kerns WP. Copperhead envenomations in the Carolinas. J Toxicol - Clin Toxicol 2003;41:29–35.
DOI: https://doi.org/10.1081/CLT-120018268.
12. Knudsen C, Laustsen AH. Recent advances in next generation snakebite antivenoms. Trop Med Infect Dis 2018;3.
DOI: https://doi.org/10.3390/tropicalmed3020042.
13. Kanjanabuch T, Sitprija V. Snakebite Nephrotoxicity in Asia. Semin Nephrol 2008;28:363–72.
DOI: https://doi.org/10.1016/j.semnephrol.2008.04.005.
14. Chugh KS. Snake-bite-induced acute renal failure in India. Kidney Int 1989;35:891–907.
DOI: https://doi.org/10.1038/ki.1989.70.
15. Greene S, Bush SP. What percentage of snake species are venomous? Medscape 2020.
https://www.medscape.com/answers/ 168828-39085/what-percentage-of-snake-species-are-venomou (accessed March 18, 2020).
16. Ratnayake I, Shihana F, Dissanayake DM, Buckley NA, Maduwage K, Isbister GK. Performance of the 20-minute whole blood clotting test in detecting venom induced consumption coagulopathy from Russell's viper (Daboia russelii) bites. Thromb Haemost 2017;117:500–7.
DOI: https://doi.org/10.1160/TH16-10-0769.
17. Palevsky PM, Liu KD, Brophy PD, Chawla LS, Parikh CR, Thakar CV, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for acute kidney injury. Am J Kidney Dis. 2013;61:649–72.
DOI: https://doi.org/10.1053/j.ajkd.2013.02.349.
18. IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp n.d.
19. Harshavardhan L, Lokesh AJ, Tejeshwari HL, Halesha BR, Metri SS. A study on the acute kidney injury in snake bite victims in a tertiary care centre. J Clin Diagnostic Res 2013;7:853–6.
DOI: https://doi.org/10.7860/JCDR/2013/5495.2957.
20. Kumar KS, Narayanan S, Udayabhaskaran V, Thulaseedharan NK. Clinical and epidemiologic profile and predictors of outcome of poisonous snake bites - an analysis of 1,500 cases from a tertiary care center in Malabar, North Kerala, India. Int J Gen Med 2018;11:209–16.
DOI: https://doi.org/10.2147/IJGM.S136153.
21. Simpson ID, Norris RL. Snakes of medical importance in India: is the concept of the "Big 4" still relevant and useful? Wilderness Environ Med 2007;18:2–9.
DOI: https://doi.org/10.1580/06-weme-co-023r1.1.
22. Bhalla G, Mhaskar D, Agarwal A. A study of clinical profile of snake bite at a tertiary care centre. Toxicol Int 2014;21:203–8.
DOI: https://doi.org/10.4103/0971-6580.139811.
23. Mallow D, Ludwig D NG. True vipers: natural history and toxinology of Old World vipers. vol. 41. Krieger Publishing Company; 2003.
DOI: https://doi.org/10.5860/choice.41-2177.
24. Tun-Pe, Phillips RE, Warrell DA, Moore RA, Tin-Nu-Swe, Myint-Lwin, et al. Acute and chronic pituitary failure resembling Sheehan's syndrome following bites by Russell's viper in Burma. Lancet (London, England) 1987;2:763–7.
DOI: https://doi.org/10.1016/s0140-6736(87)92500-1.
25. Fatah C. Pathophysiological and Pharmacological Effects of Snake Venom Components: Molecular Targets. J Clin Toxicol 2014;04.
DOI: https://doi.org/10.4172/2161-0495.1000190.

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POLYMORPHISMS OF INSULIN RECEPTOR SUBSTRATE 1 AS A RISK FACTOR FOR TYPE 2 DIABETES MELLITUS, OBESITY AND CHRONIC PANCREATITIS AMONG POPULATION OF TERNOPIL REGION

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Background. *The course of type 2 diabetes mellitus (T2DM), obesity and chronic pancreatitis (CP) in most cases is not isolated, so it requires broadening the knowledge about the pathogenetic links at their combined course. Despite significant advances in genome research, most of the genetic factors that cause development of T2DM are still unclear.*

Objective. *The aim of the study was to establish the prevalence of IRS1 gene polymorphism in the patients with T2DM as well as obesity and CP.*

Methods. *The study involved 33 patients with T2DM who were hospitalized in the endocrinology department of Ternopil University Hospital in 2019-2020 and 10 apparently healthy patients. Analysis of IRS1 gene polymorphism (SNP in the promoter region – rs2943640; gene localization 2q36.3) was performed on the basis of polymerase chain reaction data using specific primers.*

Results. *It was found that the frequencies of the genotype responsible for C/A polymorphism of IRS1 gene in T2DM, T2DM with obesity and in the combined course of T2DM with obesity and CP did not deviate significantly from the Hardy-Weinberg equilibrium ($p>0.05$). The patients with combined course of T2DM, obesity and chronic pancreatitis experienced a probable influence of genotypes C/C and C/A of IRS1 gene on the development of the studied comorbidity ($p<0.05$), which is confirmed by a probable difference in the dominant model of IRS1 gene inheritance only when T2DM was combined with obesity and CP compared to the control group ($p<0.001$).*

Conclusions. *The presence of the C allele in both homozygous and heterozygous states may increase the risk of T2DM comorbidity, obesity and CP in the population of Ternopil region.*

KEYWORDS: **type 2 diabetes mellitus; obesity; chronic pancreatitis; insulin receptor substrate 1; genes polymorphism.**

Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial genetic disease that causes significant morbidity and mortality worldwide [1]. Multimorbidity, the presence of two or more chronic diseases [2], is typical for patients with T2DM, which makes multimorbidity in this population a significant clinical issue. Nowakowska et al. found out that almost 75% of patients had at least one additional comorbidity at the time of T2DM diagnosis, and 44% – at least two comorbidities [3]. Other studies indicate diabetic complications in 90% [4], 91.4% [5], 84.6% [6], 44% [7] of patients with T2DM, which probably depends on the age and gender coverage of the respondents in the study.

The course of T2DM, obesity and chronic pancreatitis (CP) in most cases is not defined, so it requires broadening the knowledge about the pathogenetic links at their combined course [8-11]. It is obvious that T2DM is a poorly controlled epidemic that requires active research of the mechanisms of development, features of the course, methods of prevention, treatment and prevention of complications [12]. Despite significant advances in genome research, most of the genetic factors that cause development of T2DM are still unclear [13, 14]. The insulin receptor substrate (IRS) is a key central receptor in transmission of insulin signals. Several IRS polymorphisms have been identified, but the Gly to Arg 972 substitution of IRS1 probably is pathogenetically crucial in the development of T2DM [15].

Therefore, the aim of our study was to establish the prevalence of IRS1 gene polymorphism in the patients with type 2 diabetes mellitus as well as obesity and chronic pancreatitis.

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Methods

The study involved 33 patients with T2DM who were hospitalized in the endocrinology

department of Ternopil University Hospital in 2019-2020 and 10 apparently healthy patients. The distribution of groups is presented in Table 1.

There was no significant difference in age and gender between the patients of experimental groups. All patients were informed of the purpose of the clinical trial and gave written informed consent to participate in it. The privacy of the patient's identity and health has been maintained.

Verification of T2DM was performed in accordance with the recommendations of the American Diabetes Association (2019) [16]. The criteria for T2DM diagnosing were based on the rate of glycated hemoglobin (HbA1c) (≥6.5%), which was determined using the COBAS 6000 automatic biochemical analyzer (Roche Hitachi, Germany).

Verification of chronic pancreatitis (CP) was based on the Unified Clinical Protocol of Primary, Secondary (Specialized) Medical Care and Medical Rehabilitation "Chronic Pancreatitis" and the recommendations of the American Pancreatic Association [15,16].

BMI was calculated by the BMI formula = body weight (kg) / height (m^2). The data were interpreted according to the WHO recommendations: normal weight in the range of 20.0-24.9 kg/m^2; overweight (pre-obesity) – 25.0-29.9 kg/m^2; class 1 obesity – 30.0-34.9 kg/m^2; class 2 obesity – 35.0-39.9 kg/m^2 and class 3 obesity >40 kg/m^2 [17].

Inclusion criteria were: clinical, laboratory and instrumental signs of T2DM, CP and obesity, no severe increase (not more than in 3 times from the upper of norm maximum) of alpha-amylase, lipase, ALT, AST, alkaline phosphatase, gamma-glutamyltranspeptidase in the blood.

Exclusion criteria from the study were: signs of clinically significant neurological, mental, renal, hepatic, immunological, gastrointestinal, urogenital disorders, musculoskeletal lesions, skin, sense organs, endocrine (except T2DM) or hematological diseases that were uncontrolled, acute pancreatitis, unstable or life-threatening heart diseases, malignant neoplasms not in complete remission for at least 5 years, medication (drug) dependence, alcohol dependence.

Analysis of IRS1 gene polymorphism (SNP in the promoter region – rs2943640; gene localization – 2q36.3) was performed on the basis of polymerase chain reaction data using specific primers. Genomic DNA was extracted from peripheral blood leukocytes using a commercially available DNA isolation kit (QIAamp Blood DNA Mini Kit, QIAGEN, Germany). The rs2943640 IRS1 gene polymorphism was genotyped using the TaqMan real-time PCR method (Applied Biosystems, Foster City, CA, USA). Three genotypes of IRS1 polymorphism (rs2943640) were defined (C/C, C/A and A/A).

Statistical analysis of the data was performed using the STATISTICA 7.0 software. The Hardy-Weinberg equilibrium was used to verify the conformity of the genotypes of the selected samples to the general population. Comparison of the frequencies observed and expected, calculated according to the formula p2 + 2pq + q2 = 1 (the Hardy-Weinberg equilibrium), was performed using the Pearson's chi-square χ^2. In assessment of the reliability coefficient p>0.05, the null hypothesis of the samples equality was taken into the account, i.e. the correspondence of the selected sample to the general population.

Comparative analysis of frequency tables was performed using the Pearson's chi-square χ^2 and two-tailed p-value for Fisher's exact test (in cases of the expected frequencies of individual indicators not exceeding 5).

To evaluate the influence of the factor (the presence of a certain genotype or allele of the gene) on the disease incidence, calculation of the odds ratio (OR), its 95% confidence interval (CI) and the reliability coefficient p-value was used.

Results

Insulin receptor substrate (IRS) molecules are key mediators in transmission of insulin signals. Several polymorphisms have been defined in IRS genes, but only the Gly to Arg

Table 1. Characteristic features of the study groups (n=44)

Group №	Characteristics of the group	n	%
1	T2DM patients with normal body weight without chronic pancreatitis	9	22.7
2	T2DM patients with overweight / obesity without chronic pancreatitis	14	31.9
3	T2DM patients with overweight / obesity with concomitant chronic pancreatitis	10	22.7
4	Apparently healthy patients (control)	10	22.7

972 substitution of IRS-1 is pathogenically crucial in development of T2DM. However, other polymorphisms described in IRS-1 gene are associated with insulin resistance (IR) in T2DM. The frequency distribution of polymorphic genotypes of IRS1 gene (rs2943640) and verification of conformity with the Hardy-Weinberg population equilibrium were performed in the experimental and control groups. It was found out that the frequencies of the genotype responsible for C/A polymorphism of IRS1 gene in T2DM, T2DM with obesity and at the combined course of T2DM with obesity and CP did not deviate significantly from the Hardy-Weinberg equilibrium (p>0.05), while in the control group the selected sample did not correspond to the general population (Table 2).

The corresponding frequencies for the genotypes of IRS1 gene were as follows: 66.7% for C/A and 33.3% for A/A in the experimental group 1; 21.4% for C/A, 64.3% for C/A and 14.3% for A/A in the group 2; 70.0% for C/A, 10.0% for C/A and 20.0% for A/A in the group 3 and 100.0% for C/A in the control group (Table 3).

The frequencies of alleles of IRS1 gene in the patients involved in the study are presented in Table 2. In the patients with T2DM the allele C predominated, in the patients with T2DM + obesity + CP – allele A, while in the patients with T2DM + obesity and in the control group, the C allele and the A allele were almost equal.

The obtained results, presented in Table 4, indicate the absence of a statistically significant relationship between the factor (presence of alleles C or A) and the disease incidence (p>0.05).

Evaluation of the reliability coefficient in the analysis of odds ratio showed probable influence of the genotypes C/C and C/A of the IRS1 gene on development of T2DM combined with

Table 2. Polymorphism of IRS1 genes (rs2943640) according to the Hardy-Weinberg equilibrium in T2DM and its comorbidity

Genotypes		T2DM (Group 1)		T2DM+obesity (Group 2)		T2DM+obesity+CP (Group 3)		Control	
		Expected	Present	Expected	Present	Expected	Present	Expected	Present
Polymorphism of IRS1 gene (rs2943640)									
Homozygotes that are common	C/C	1	0	4,0	3	6.4	7	2.5	0
Heterozygotes	C/A	4	6	7.0	9	3.2	1	5	10
Homozygotes that are rare	A/A	4	3	3.0	2	0.4	2	2.5	0
χ^2, p		χ^2=2.25; p>0.05		χ^2=1.20; p>0.05		χ^2=2.45; p>0.05		χ^2=10.00; p<0.01*	

Table 3. Frequency of the alleles of the IRS1 genes (rs2943640) in T2DM and its comorbidity

Frequency of alleles	T2DM (group 1)		T2DM+obesity (group 2)		T2DM+obesity+CP (group 3)		Control	
	n	%	n	%	n	%	n	%
Polymorphism of IRS1 gene (rs2943640)								
Allele C	6	33.33	15	53.57	16	80.00	10	50.00
Allele A	12	66.67	13	46.43	4	20.00	10	50.00
p_F (patients/control)	p_F=0.342		p_F=0.999		p_F=0.096		–	

Table 4. The odds ratio for alleles in different study groups in T2DM and its comorbidity

Group	IRS1 gene (rs2943640)					
	Allele C			Allele A		
	OR	95 % CI	p	OR	95 % CI	p
T2DM (Group 1)	0.50	0.13-1.86	>0.05	2.00	0.54-7.45	>0.05
T2DM+obesity (Group 2)	1.15	0.37-3.64	>0.05	0.87	0.27-2.73	>0.05
T2DM+obesity+CP (Group 3)	4.00	0.98-16.27	>0.05	0.25	0.06-1.02	>0.05

obesity and CP (p<0.05) (Table 5). This is confirmed by a probable difference in the dominant model of IRS1 gene inheritance only in the group with combination of T2DM as well as obesity and CP compared with the control group (reliability coefficient for the chi-square p<0.001). Thus, the presence of the C allele in both homozygous and heterozygous states may increase the risk of T2DM comorbidity, obesity and CP (Table 6).

It should be noted that in recessive models of IRS1 gene inheritance in T2DM, T2DM + obesity and T2DM + obesity + CP no significant differences were established compared with the control group, but there was also a tendency to increase in the probability of development of these diseases in the presence of C allele (Table 7).

Discussion

It is established that genes are crucial in development of T2DM. Researchers have suggested the interaction between many genetic factors and environmental factors that

Table 5. The odds ratio for genotypes in different study groups in T2DM and its comorbidity

Group	Genotype					
	Polymorphism of IRS1 gene (rs2943640)					
	CC		CA		AA	
	OR	95 % CI	OR	95 % CI	OR	95% CI
T2DM (Group 1)	1.11	0.02-61.38	0.09	0.01-2.00	11.31	0.50-256.21
T2DM+obesity (Group 2)	6.39	0.29-138.94	1.73	0.03-99.96	4.20	0.18-97.55
T2DM+obesity+CP (Group 3)	71.40*	3.00-1696.84	0.002*	0.001-0.13	6.18	0.26-146.79

*Note. * – p<0.05.*

Table 6. Dominant model of IRS1 gene inheritance (rs2943640) in T2DM and its comorbidity

Genotypes	Experimental group %	Control %	p_F	OR	95% CI	p
T2DM (Group 1)						
C/C	0	0	–	1.11	0.02–61.38	>0.05
C/A+A/A	100.00	100.00		0.90	0.02–50.25	>0.05
T2DM+obesity (Group 2)						
C/C	21.43	0	0.239	6.39	0.29–138.94	>0.05
C/A+A/A	78.57	100.00		0.16	0.01–3.40	>0.05
T2DM+obesity+CP (Group 3)						
C/C	80.00	0	<0.001*	71.40	3.00–1696.84	0.008*
C/A+A/A	20.00	100.00		0.01	0.001–0.33	0.008*

*Note. * – statistically significant results.*

Table 7. Recessive model of IRS1 inheritance (rs2943640) in T2DM and its comorbidity

Genotypes	Experimental group %	Control %	p_F	OR	95 % CI	p
T2DM (Group 1)						
C/C+C/A	66.67	100.00	0.087	0.09	0.01–2.00	>0.05
A/A	33.33	0		11.31	0.50–256.21	>0.05
T2DM+obesity (Group 2)						
C/C+C/A	85.71	100.00	0.493	0.24	0.01–5.53	>0.05
A/A	14.29	0		6.18	0.26–146.79	>0.05
T2DM+obesity+CP (Group 3)						
C/C+C/A	80.00	100.00	0.474	0.16	0.01–3.85	>0.05
A/A	20.00	0		6.18	0.26–146.79	>0.05

contribute to the disease development [20]. However, there are only isolated data in the literature on the role of IRS1 polymorphism (rs2943640) in increased susceptibility to T2DM. Thus, Langenberg C et al. presented the results of the InterAct study, which proved the effect of C allele of IRS1 gene polymorphism (rs2943640) on the risk of T2DM development [21]. In the study prepared by Liu J and et al. 8 SNPs associated with T2DM were found, including rs2943640 variant of IRS1 gene, which was also associated with body mass index [22].

A study of the physical activity genetics made by Loos RJ et al., which included IRS1 (rs2943640) among the studied genes, showed an increased interaction of the gene with the risk of diabetes, in particular, a dependence on genetic susceptibility to insulin resistance [23]. Another interesting result of this study is the established data on the higher genetic risk of T2DM in individuals with the highest level of physical activity that is consistent with the large-scale study of Langenberg et al. (24), in which the predicted effect of T2DM genetic risk was the strongest among young, lean, and physically active individuals [21]. It should be noted that according to Karaderi T. et al., rs2943640 variant of IRS1 gene is associated with decreased BMI [24]. Our results prove the effect of C allele of IRS1 gene (rs2943640) on the increased susceptibility to the combination of T2DM+obesity+CP, but there is no probable effect of the factor (alleles C and A) on development of only T2DM and T2DM+obesity. There are no data in the literature on the role of IRS1 gene (variant rs2943640) in increased susceptibility to CP.

There are some limitations in this study that need to be considered when interpreting its results: the sample size is too small, so it is difficult to find significant relationships between the data; inclusion into the experimental groups only of patients with comorbidity T2DM+obesity and T2DM+obesity+CP; patients were not randomly selected generating a potential selection bias. Therefore, we cannot exclude the hypothesis that the evaluated patients do not represent the whole population of the patients with comorbid T2DM, but these results are the first step in finding genes for increased susceptibility to the studied pathology, they reflect a more heterogeneous real-world population characteristic in clinical practice.

Conclusions

The patients with combined course of T2DM, obesity and chronic pancreatitis have a probable influence of genotypes C/C and C/A of IRS1 gene on development of the studied comorbidity ($p<0.05$) that is confirmed by a probable difference in the dominant model of IRS1 gene inheritance only when T2DM is combined with obesity and CP compare to the control group ($p<0.001$). Thus, the presence of the C allele in both homozygous and heterozygous states may increase the risk of T2DM comorbidity, obesity and CP in the population of Ternopil region.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Hevko U.P. – methodology, software, validation, formal analysis, investigation, resources, data curation, visualization, writing – original draft, writing – reviewing and editing.

Marushchak M.I. – conceptualization, visualization, supervision, writing – reviewing and editing.

ПОЛІМОРФІЗМ СУБСТРАТУ ІНСУЛІНОВИХ РЕЦЕПТОРІВ 1 ТИПУ ЯК ФАКТОР РИЗИКУ РОЗВИТКУ ЦУКРОВОГО ДІАБЕТУ 2 ТИПУ, ПОЄДНАНОГО З ОЖИРІННЯМ ТА ХРОНІЧНИМ ПАНКРЕАТИТОМ У НАСЕЛЕННЯ ТЕРНОПІЛЬСЬКОЇ ОБЛАСТІ

У.П. Гевко, М.І. Марущак

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО МОЗ УКРАЇНИ, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Перебіг цукрового діабету 2 типу (Т2DM), ожиріння та хронічного панкреатиту (СР) в більшості випадків не є ізольованим, тому потребує поглиблення знань стосовно патогенетичних*

ланок при їх поєднаному перебігу. Незважаючи на значні успіхи у дослідженнях геному, більшість генетичних факторів, що спричинюють розвиток T2DM, залишаються невизначеними.

Мета дослідження *– встановити поширення поліморфізму гена субстрату інсулінових рецепторів 1 типу (IRS1) у хворих на T2DM у поєднанні з ожирінням та СР.*

Методи дослідження. *У дослідження було включено 33 хворих на T2DM, які перебували на стаціонарному лікуванні в ендокринологічному відділенні Тернопільської університетської лікарні у 2019-2020 рр. та 10 практично здорових осіб. Аналіз поліморфізму гена IRS1 (SNP у промоторному регіоні – rs2943640; генна локалізація 2q36.3) проведено на підставі даних полімерної ланцюгової реакції з використанням специфічних праймерів.*

Результати. *Встановлено, що частоти генотипу, що відповідає за C/A поліморфізм гена IRS1 при T2DM, T2DM з ожирінням та при поєднаному перебігу T2DM з ожирінням та СР суттєво не відхилялися від рівноваги Харді–Вайнберга (p>0,05). У хворих на поєднаний перебіг цукрового діабету 2 типу, ожиріння та хронічного панкреатиту виявляється вірогідний вплив генотипів C/C та C/A гена IRS1 на розвиток досліджуваної коморбідності (p<0,05), що підтверджується вірогідною відмінністю у домінантній моделі успадкування IRS1 гену тільки при поєднанні T2DM з ожирінням та СР порівняно з групою контролю (p<0,001).*

Висновки. *Наявність алелі С як в гомозиготному, так і в гетерозиготному станах може підвищувати ризик виникнення коморбідності T2DM, ожиріння та СР.*

КЛЮЧОВІ СЛОВА: **цукровий діабет 2 типу; ожиріння; хронічний панкреатит; субстрат інсулінових рецепторів 1 типу; поліморфізм гена.**

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References

1. Roy S, Sherman A, Monari-Sparks MG, Schweiker O, Jain N, Etty Sims E, et al. Association of comorbid and metabolic factors with optimal control of type 2 diabetes mellitus. N Am J Med Sci. 2016; 8(1):31-9.

DOI: https://doi.org/10.4103/1947-2714.175197

2. Jani BD, Hanlon P, Nicholl BI, McQueenie R, Gallacher KI, Lee D, et al. Relationship between multimorbidity, demographic factors and mortality: findings from the UK Biobank. BMC Med. 2019; 17(1):74.

DOI: https://doi.org/10.1186/s12916-019-1305-x

3. Nowakowska M, Zghebi SS, Ashcroft DM, Buchan I, Chew-Graham C, Holt T, et al.The comorbidity burden of type 2 diabetes mellitus: patterns, clusters and predictions from a large English primary care cohort. BMC Medicine. 2019;17:145.

DOI: https://doi.org/10.1186/s12916-019-1373-y

4. Teljeur C, Smith SM, Paul G, Kelly A, O'Dowd T. Multimorbidity in a cohort of patients with type 2 diabetes. Eur J Gen Pract. 2013;19 (1):17-22.

DOI: https://doi.org/10.3109/13814788.2012.714768

5. Lonso-Morán E, Orueta JF, Esteban JIF. The prevalence of diabetes-related complications and multimorbidity in the population with type 2 diabetes mellitus in the Basque Country. BMC Public Health. 2014;14: 1059.

DOI: https://doi.org/10.1186/1471-2458-14-1059

6. Luijks H, Schermer T, Bor H, Weel C, Lagro-Janssen T, Biermans M, et al. Prevalence and incidence density rates of chronic comorbidity in type 2 diabetes patients: an exploratory cohort study BMC Med. 2012; 10 (1): 128.

DOI: https://doi.org/10.1186/1741-7015-10-128

7. Struijs JN, Baan CA, Schellevis FG, Westert GP, van den Bos GA. Comorbidity in patients with diabetes mellitus: impact on medical health care utilization. BMC Health Serv Res. 2006; 6: 84.

DOI: https://doi.org/10.1186/1472-6963-6-84

8. Li X, Guo X, Ji H, Niu J, Gao P. Relationships between metabolic comorbidities and occurrence, severity, and outcomes in patients with acute pancreatitis: A narrative review. Biomed Res Int. 2019 Oct 7;2019:2645926.

DOI: https://doi.org/10.1155/2019/2645926

9. Samsonova NG, Zvenigorod LA. Pancreas and metabolic syndrome. Experimental and clinical gastroenterology. 2011; 11: 68-72.

10. American Diabetes Association. 4. Comprehensive medical evaluation and assessment of comorbidities: Standards of Medical Care in Diabetes–2019. Diabetes care. 2019 Jan 1;42(Supplement 1):S34-45.

DOI: https://doi.org/10.2337/dc19-S004

11. Antonyshyn IV, MI Marushchak, OV Denefil. [State of lipid peroxidation in experimental diet-induced alimentary obesity]. Medical Chemistry. 2014; 16 (3): 61-5. (In Ukrainian)

12. Hevko U, Kozak K, Krynytska I, Marushchak M. Diagnostic value of a complete blood count in type 2 diabetes mellitus and comorbidities. Balkan Medical Union. 2020; 55(4): 601-7.

DOI: https://doi.org/10.31688/ABMU.2020.55.4.06

13. Plengvidhya N, Chanprasert C, Chongjaroen N, et al. Impact of KCNQ1, CDKN2A/2B, CDKAL1, HHEX, MTNR1B, SLC30A8, TCF7L2, and UBE2E2 on risk of developing type 2 diabetes in Thai population. BMC Med Genet. 2018; 19:93.

DOI: https://doi.org/10.1186/s12881-018-0614-9

14. Musiienko VA, Marushchak MI. [Type 2 diabetes and thyroid disease: finding common mechanisms]. Vìsn. med. bìol. dosl.. 2020; 1: 74-82. (In Ukrainian).

DOI: https://doi.org/10.11603/bmbr.2706-6290.2020.1.11006

15. Yousef AA, Behiry EG, Allah WMA, Hussien AM, Abdelmoneam AA, Imam MH, Hikal DM. IRS-1 genetic polymorphism (r.2963G>A) in type 2 diabetes mellitus patients associated with insulin resistance. Appl Clin Genet. 2018 Sep 28;11:99-106.

DOI: https://doi.org/10.2147/TACG.S171096

16. American Diabetes Association. Cardiovascular disease and risk management: Standards of Medical Care in Diabetes—2019. Diabetes care. 2019 Jan 1;42(Supplement 1):S103-23.

DOI: https://doi.org/10.2337/dc19-S010

17. Conwell DL, Lee LS, Yadav D, Longnecker DS, Miller FH, Mortele KJ, et al. American Pancreatic Association Practice Guidelines in Chronic Pancreatitis: evidence-based report on diagnostic guidelines. Pancreas. 2014;43(8):1143-62.

DOI: https://doi.org/10.1097/MPA.0000000000000237

18. Order of the Ministry of Health of Ukraine № 638 dated 10.09.2014 https://zakononline.com.ua/documents/show/35092___35092.

19. Body Mass Index: Considerations for Practitioners / https://www.cdc.gov/obesity/downloads/bmiforpactitioners.pdf.

20. Chen J, Meng Y, Zhou J, Zhuo M, Ling F, Zhang Yu,et al. Identifying candidate genes for type 2 diabetes mellitus and obesity through gene expression profiling in multiple tissues or cells. J Diab Res. 2013:9.

DOI: https://doi.org/10.1155/2013/970435

21. Langenberg C, Sharp SJ, Franks PW, et al. Gene-lifestyle interaction and type 2 diabetes: the EPIC interact case-cohort study. PLoS Med. 2014 May;11 (5):e1001647.

DOI: https://doi.org/10.1371/journal.pmed.1001647

22. Liu J, Rutten-Jacobs L, Liu M, Markus HS, Traylor M. Causal impact of type 2 diabetes mellitus on cerebral small vessel disease: A mendelian randomization analysis. Stroke. 2018 Jun;49 (6): 1325-31.

DOI: https://doi.org/10.1161/STROKEAHA.117.020536

23. Loos RJ, Hagberg JM, Pérusse L, et al. Advances in exercise, fitness, and performance genomics in 2014. Med Sci Sports Exerc. 2015 Jun;47 (6):1105-12.

https://doi.org/10.1249/MSS.0000000000000645

24. Karaderi T, Drong AW, Lindgren CM. Insights into the Genetic Susceptibility to Type 2 Diabetes from Genome-Wide Association Studies of Obesity-Related Traits. Curr Diab Rep. 2015;15 (10):83.

DOI: https://doi.org/10.1007/s11892-015-0648-8

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ENDOTHELIAL DYSFUNCTION AND ITS MANAGEMENT IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION COMBINED WITH METABOLIC SYNDROME

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Background. *Oxidative stress, endothelial dysfunction, dyslipidemia and low-grade inflammation induce the disorders of energy metabolism and ischemic damage to cardiomyocytes. It is an essential issue in pathogenesis of acute coronary syndrome/myocardial infarction (ACS/MI).*

Objective. *The aim of the study was to improve the existing pharmacological therapy in patients with ACS/MI combined with metabolic syndrome (MS).*

Methods. *The study enrolled 95 patients with acute myocardial infarction. The patients were divided into 2 groups depending on concomitant metabolic syndrome. All groups were divided to subgroups, where patients received typical standard treatment according to the Ukrainian unified and modified treatment regimen with addition of L-arginine and L-carnitine.*

Results. *In 79.2% of patients with ACS/MI + MS the course of underlying disease was associated with various complications: pericarditis epistenocardica was diagnosed in 39.8% of patients; arrhythmias were present in 35.5% of patients of the main group; left ventricular aneurysm was present in 15.9% of patients. At the same time, significant changes in the indicators of vascular endothelial function in patients with ACS (MI) were revealed (the level of endothelin-1 in the blood plasma was in 2.1 times higher than the reference norm) that was the justification for inclusion of L-arginine and L-carnitine in the complex therapy of comorbid patients.*

Conclusions. *The multi-modality treatment with inclusion of L-arginine and L-carnitine facilitated restoration of energy supply of myocardial contractility, endothelial function of blood vessels, and antioxidant protection of the body and ultimately resulted in a more favorable course of this comorbid problem.*

KEYWORDS: **myocardial infarction; metabolic syndrome; endothelial dysfunction; L-arginine; L-carnitine.**

Introduction

The rupture of the atheromatous plaque and formation of coronary clot, which causes progressive stenosis, is considered the main cause of acute myocardial infarction (MI). However, the recently discussed pathogenetic factors include peroxide stress and endothelial dysfunction, dyslipidemia and low-intensity systemic inflammation underlying the disorders of energy metabolism and ischemic damage to cardiomyocytes. The aforementioned pathogenetic mechanism behind the development of acute coronary syndrome/myocardial infarction (ACS/MI) provides a possibility of management of these pathogenetic processes with metabolic and cytoprotective pharmacological therapy. In addition to that, the main risk factors for coronary heart disease and myocardial infarction as its life-threatening manifestation are obesity, hypertension, dyslipidemia, insulin resistance and diabetes, which as a whole can be components of metabolic syndrome (MS), the latter diagnosed in 28-35% patients with myocardial infarction [1, 4, 13]. The causes of MS, including insulin resistance, hyperinsulinemia and chronic inflammation simultaneously trigger and maintain high levels of atherogenesis, endothelial dysfunction and evoke coronary plaque instability as well as processes of thrombogenesis [6, 7, 8]. That is why a differentiated approach to treatment of patients with ACS/MI (with due consideration for comorbidities) is a priority at present stage. Both L-carnitine and L-arginine have been proved to be very promising metabolic drug products, which occur naturally in human body. They manifest their effects as active regulators of intermediary metabolism and energy-supplying processes [7, 12, 14]. However, their main physiological role includes

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regulation of functional state of blood vessels and maintaining adequate microcirculation in the organs and tissues of the body [9, 11]. The above facts have become a rationale for studies of clinical efficacy and possibilities for correction of metabolic and endothelial disorders in patients with ACS/MI.

The aim of the study was to improve the existing therapeutic programs for ACS/MI combined with metabolic syndrome (MS).

Methods

The study enrolled 95 patients with acute myocardial infarction. The patients were divided into 2 groups depending on whether they had metabolic syndrome. The main study group involved 53 patients with ACS/MI+MS. The control group included 42 patients with ACS/MI, who were not diagnosed with MS; 32 patients in the main group and 22 patients in the control group received standard treatment for myocardial infarction according to the Unified Protocols of the Ministry of Health of Ukraine [5]. Other 21 patients in the main group and 20 patients in the control group received a modified treatment regimen with addition of L-arginine 4.2 g and L-carnitine 2.0 g supplied as solution for infusions 100 mL administered intravenously once a day as a 5-day course. Most of study participants were males (87.5%) of productive age, 57.54±8.02 years old on average.

The diagnosis of acute MI was verified according to the 2017 ESC Guidelines [2] in the presence of a typical anginal attack, MI-specific ECG changes with time (reciprocal ST displacement) and the signs of resorption-necrosis syndrome. The diagnosis was confirmed by means of laboratory tests, ECG and imaging tests. Quantitative determination of troponin T was performed using an electro chemiluminescent biochemical analyzer Elecsys 2010 by Roche/Hitachi (Switzerland). Troponin test results above the reference value of 14.0 ng/mL were assessed as positive.

Patient assessments included general clinical examination, laboratory tests (hematology, MB fraction of creatine phosphokinase (CPK-MB), troponin T, oxygen saturation of arterial blood (SpO2), ECG in 12 standard leads, etc. and B-mode cardiac ultrasound imaging on Aloka SSD – 2000 unit (USA) with determination of linear and volumetric parameters of the left ventricle, as well as global (assessed by ejection fraction, EF) and local myocardial contractility using the Simpson's biplane method. The diastolic function of the left ventricle was assessed by the isovolumic relaxation time (IVRT) of the left ventricle, delay time for early left ventricular filling (DT), maximal early filling velocity (E) and maximal atrial systole filling velocity of the left ventricle (A). Plasma level of endothelin-1 (ET-1) was evaluated by the ELISA kits by Amersham Pharmacia Biotech; the stable NO metabolites content was determined by the Griess reagent reaction [2]. The number of nitrites was measured using a calibration graph. The above-mentioned tests were repeated on day 14 and day 28 and tracked as they changed with time. Statistical data analysis was performed using the STATISTICA statistical package (by StatSoft, USA, v 6.0).

Results

Uncomplicated course of myocardial infarction was established in 17 patients (40%) of the control groups. In 42 patients with MI+MS (79.2%), the course of their underlying disease was accompanied by various complications. Thus, the patients of the test group were substantially more likely to develop pericarditis epistenocardica (21 (39.8%) patients) than those in the control group (8 (19%) patients). Conduction disorders or arrhythmias manifested as paroxysmal tachyarrhythmia, transient atrioventricular block and bundle branch blocks; extrasystole arrhythmia were also more frequent in the patients of the main group (78.5%) and significantly less frequent in the patients with MI without MS (52%). These results are presented in Fig. 1.

Acute left ventricular failure accompanied the course of MI in all patients of both groups; however, Killip I and Killip II acute heart failure (according to the Killip-Kimball Classification, 1972) was substantially more frequent in the patients of the control group. Impaired systolic and diastolic functions of the left ventricle were simultaneously observed in the patients of the main group and the control group. That is, there was a significantly more significant reduction in myocardial contractility (EF=42.12±1.13%) compared to the control group (EF=48.23±1.16%). These changes were closely related to the more pronounced processes of left ventricular remodeling during the acute period of myocardial infarction in the patients of the main group. In particular, the increase in left ventricular internal diastolic dimension (the left ventricular end-diastolic diameter, LVEDD) was 5.62±0.21 cm in the main group and 4.71±0.24 cm in the control group ($p<0.05$). The developing diastolic dysfunction was suggested by changes in the

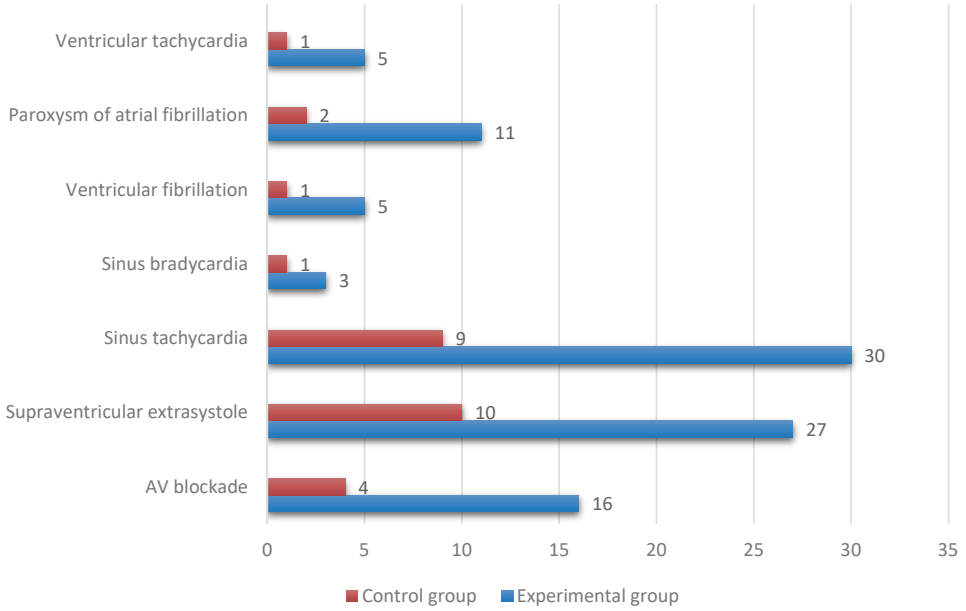

Fig 1. Frequency of arrhythmias and conduction disorders in the patients with acute coronary syndrome (myocardial infarction).

isovolumic relaxation times (IVRT) in the main group (63.23±0.41 msec) and the control group (68.12±0.36 msec), as well as by delay time of early diastolic transmitral flow (DT) in the compared groups (178.11±0.54 msec and 182.43±0.37 msec, respectively).

After completion of a standard MI treatment, the patients of the control group experienced significant improvement of hemodynamic parameters. Relatively, there was an increase in left ventricular ejection fraction, a reduction in LVEDD and improvement in left ventricular diastolic function parameters, i.e. IVRT and DT, compared to the MI+MS patients ($p<0.05$). However, the patients with myocardial infarction combined with MS experienced more frequent complications in a setting of standard treatment. This is because of special characteristics of carbohydrate metabolism in the patients with MS, which are manifested by insulin resistance and compensatory hyperinsulinemia (which activates the sympathoadrenal system), as well as by a combination of this pathogenetic mechanism with other pathogenetic factors of ischemia (an increase in lipid peroxidation and endothelial dysfunction) that is accompanied by a more prolonged activation of oxidative processes and inhibited activity of enzymes in the antioxidant protection system. The abnormal processes in the heart enhanced causing a significant reduction of myocardial contractility and substantial changes in electrolyte stability of the heart that contributed to life-threatening disorders of rhythm and conductivity against the backdrop of unstable hemodynamics [9, 11].

As a consequence, L-arginine and L-carnitine are promising treatments. In addition to their pronounced metabolic and energy-producing activity, these substances restore endothelial function of blood vessels, exert antioxidant effects and prevent irreversible ischemic and reperfusion damage, limit the zone of myocardial necrosis, improve cellular adjustment to functioning under hypoxia and inhibit abnormal cardiac remodeling. One of the mechanisms behind such therapeutic effect is the ability of L-arginine and L-carnitine to inhibit the formation of radicals during breakdown of fatty acids thereby reduce the damaging effects of peroxidation byproducts on the functional status of ion channels in the cells [1, 3, 17].

Thus, the results of this study have demonstrated that a multi-modality therapeutic program with inclusion of L-arginine and L-carnitine in the patients of the main group had a substantial effect on restoration of systolic-diastolic function of the heart: the parameters of systolic and diastolic function of the heart returned to normal already within 28 days of treatment. That is, the linear dimensions of cardiac cavities changed insignificantly; thus, we may assume that the positive changes in EF, LVEDD, IVRT and DT compared to their respective baseline levels were driven by remodeling and restoration of functional status in zones of myocardial ischemia and hibernation [10].

The changes in the parameters of endothelial function of blood vessels were also assessed in the patients with ACS/MI as part of the study. The baseline levels of these parameters did not differ in between. However, they were significantly changed compared to the reference values. Thus, plasma levels of endothelin-1 in the patients with ACS/MI at hospitalization were in 2.1 times above the reference value (0.96±0.04 ng/mL and 0.46±0.03 ng/mL, respectively). There were no significant changes in endothelin-1 (ET-1) activity immediately after emergency angioplasty of the coronary vessel and its stenting. That is, the levels of endothelin were substantially reduced in the control group patients who received per-protocol pharmacological treatment. However, by the end of the in-patient treatment period, the levels of endothelin have not reached those of healthy individuals (0.66±0.06; $p>0.05$).

In contrast, the patients of the test group, whose multi-modality treatment included the course of therapy with L-arginine and L-carnitine during the next 10 days of the in-patient treatment period, had a 33.3% reduction in ET-1 activity ($p<0.05$), and after one month of treatment its plasma levels significantly decreased by additional 43% and reached the level of healthy individuals (0.52±0.05 ng/mL; $p>0.05$). Hence, the use of combination drug therapy with inclusion of L-arginine and L-carnitine in patients with ACS/MI provided for a rapid (over 10 days) reduction and complete restoration (over 28 days) of plasma endothelin-1 levels in these patients. Along with changes in endothelin-1 activity in patients with ACS/MI during exacerbation of the disease, there was an abrupt reduction in metabolites of nitric oxide (NOε=17.75±0.42 μmol/L, the normal level of 36.92±0.37 μmol/L), which could suggest pronounced microcirculatory disorders in these patients.

Thus, there was an almost two-fold baseline decrease in plasma levels of nitrates and nitrites in the patients of both groups, i. e. their total plasma levels were reduced by 45.0%. However, conventional per-protocol treatment did not lead to complete restoration of endothelial function of blood vessels in this group of patients with ACS/MI; the total plasma level of metabolites of nitric oxide in these patients was by 19% below the reference value ($p<0.05$). At the same time, the use of combination drug therapy with inclusion of L-arginine and L-carnitine had a substantial effect on plasma levels of nitrites and nitrates in the patients of the test group. The levels of these substances were significantly increased already before 10 days of treatment; after completion of the in-patient phase of combination drug therapy they reached the reference level.

Discussion

Therefore, since patients with ACS/MI + MS were diagnosed with pronounced baseline changes of morphological and functional parameters of the heart, post-infarction remodeling with compromised systolic and diastolic functions of the heart, as well as development of heart failure and endothelial dysfunction (which were retained immediately after emergency percutaneous procedures), these findings provided a rationale for inclusion of parenteral L-arginine and L-carnitine to the per-protocol therapeutic program. Standard therapy was established to lack sufficient hemodynamic efficacy in this patient cohort. Only enhancing standard therapy by adding L-arginine and L-carnitine caused significant EF and DT increases, LVEDD reduction and E/A ratio reduction and improved post-infarction cardiac remodeling, ultimately evident as a significant improvement of EF, myocardial contractility and diastolic dysfunction. In our opinion, the positive effect of the suggested treatment for inotropic heart function and a significant reduction in incidence and severity of reperfusion arrhythmias were achieved precisely by means of the cardiometabolic effect of L-carnitine, which, as reported by many researchers, is significant in energy metabolism in the myocardium by transferring free fatty acids from the cytosol inside the mitochondria thereby ensuring bio-availability of the high-energy substrate for oxidative metabolism in the cardiomyocyte [8, 16]. In addition, by facilitating oxidation of long-chain fatty acids and by modulating the CoA/CoA-SH ratio, this compound is taking part in binding of acyl residues in peroxisomes and mitochondria and has a positive effect on amino acid metabolism by assimilating the pool of free radical compounds. This ensures stabilization of organelles and cellular membranes and prevents accumulation of fatty acid esters in the cytoplasm of cardiomyocytes, which may lead to fatal ventricular arrhythmias [15]. During the study it was also established that patients of the test group, whose multi-modality treatment included additional L-arginine and L-carnitine, had significantly lower ET-1 activity, while plasma levels of nitric oxide metabolites increased rea-

ching the levels of healthy individuals (p>0.05). In other words, such multi-modality treatment in patients with ACS/MI contributed to a rapid and complete recovery of the study parameters of endothelial function of blood vessels. The treatment outcomes are associated with the use of L-arginine as a principal substrate for the synthesis of nitric oxide. The fundamental physiological role of nitric oxide is to regulate the functional status of blood vessels and to provide an adequate level of microcirculation in organs and tissues of the body [9, 11, 14].

Thus, the conclusion may be drawn that the presence of a concomitant metabolic syndrome has a substantial impact on the course of acute myocardial infarction and is accompanied by a significantly higher complication rate. These patients have more severe disorders in the lipid peroxidation system and a reduced activity of antioxidant defense, which disrupts endothelial functions of blood vessels and causes deterioration of microcirculation. At the same time, significant reductions in systolic and diastolic functions of the myocardium have been observed in patients with MI+MS as a result of impaired remodeling of cardiac chambers and changes in their linear and geometric parameters. The combination treatment of MI+MS patients with inclusion of L-arginine and L-carnitine facilitates restoration of energy supply of myocardial contractility, endothelial function of blood vessels and antioxidant defense of the body, which results in a more favorable course of this comorbid problem.

Conclusions

The patients with myocardial infarction combined with metabolic syndrome were likely to develop disorders of central and peripheral hemodynamics and endothelial function of blood vessels. These circumstances significantly exaggerate the clinical course of the underlying morbidity, additionally enhance the disorders of systolic and diastolic cardiac function and cause more frequent complications of ACS/MI. A comprehensive therapeutic program for patients with ACS/MI+MS enhanced by inclusion of L-arginine and L-carnitine facilitates restoration of endothelial function of blood vessels and antioxidant defense of the body and increases the energy supply of cardiomyocytes, which is accompanied by improved myocardial contractility.

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Conflict of Interests

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ЕНДОТЕЛІАЛЬНА ДИСФУНКЦІЯ ТА ШЛЯХИ ЇЇ КОРЕКЦІЇ У ХВОРИХ НА ГОСТРИЙ ІНФАРКТ МІОКАРДА В ПОЄДНАННІ З МЕТАБОЛІЧНИМ СИНДРОМОМ.

Ястремська І.О.

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Окислювальний стрес, дисфункція ендотелію, дисліпідемія та низькоінтенсивне запалення спричиняють порушення енергетичного обміну та ішемічне ураження кардіоміоцитів, що є важливою ланкою у патогенезі гострого коронарного синдрому / інфаркту міокарда (ГКС / ІМ).*

Мета дослідження *– удосконалити існуючі лікувальні програми ГКС(ІМ) у поєднанні з метаболічним синдромом (МС).*

Методи. *У дослідженні взяли участь 95 пацієнтів з гострим інфарктом міокарда. Пацієнтів розподілили на 2 групи залежно від наявності супутнього метаболічного синдрому. Усі групи були розділені на підгрупи, де пацієнти отримували стандартне протокольне лікування згідно Уніфікованого протоколу МОЗ України та модифікована схеми лікування з додаванням L-аргініну та L-карнітину.*

Результати дослідження. *У хворих на ІМ із супутнім МС основне захворювання перебігало з різними ускладненнями у 79,2 %: епістенокардитичний перикардит був у 39,8 %; порушення ритму або*

провідності наявні у 35,5 %; аневризма лівого шлуночка – у 15,9 % хворих. Одночасно виявлено суттєві зміни у показниках ендотеліальної функції судин у хворих на ГКС(ІМ), що стало обґрунтуванням для включення в комплексну терапію коморбідних хворих курсу L-аргініну та L-карнітину.

Висновки. *Комплексне лікування хворих на ІМ в поєднанні з МС із включеням L-аргініну та L-карнітину сприяло відновленню енергозабезпечення скоротливості міокарда, ендотеліальної функції судин, антиоксидантного захисту організму, що в результаті забезпечувало більш сприятливий перебіг даної коморбідної патології.*

КЛЮЧОВІ СЛОВА: **інфаркт міокарда; метаболічний синдром; ендотеліальна дисфункція; L-аргінін; L-карнітин.**

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References

1. Batushkin VV. [Cytoprotection in acute myocardial infarction after the discovery of infarct-dependent artery: new horizons]. Liky Ukrainy. 2017;8(214):33–40. (In Ukrainian)

2. Thiele H, Desch S, de Waha S. Acute myocardial infarction in patients with ST-segment elevation myocardial infarction: ESC guidelines 2017. Herz. 2017 Dec 1;42(8):728-38.

3. Parkhomenko OM, Kozhukhov SN, Lutay YaM. [Rationale and design of multicenter randomized study PROTECT – study of the effectiveness and safety of quercetin in patients with acute myocardial infarction]. Ukrainian Journal of Cardiology. 2016;(3): 31–6. (In Ukrainian)

4. Selyuk MM, Kozachok MM, Levkin IM, Selyuk OV. Selection of the optimal combination of metabolic drugs for the treatment of patients with cardiovascular pathology. Family Medicine. 2017; 2(70):60–4. (In Ukrainian)

5. Acute coronary syndrome with ST segment elevation: Unified clinical protocol of emergency, primary, secondary and tertiary care. – Ministry of Health of Ukraine. 2017: 78. (In Ukrainian)

6. Shved MI, Tsuglevich LV, Geryak SM. Clinical efficacy of cardiocytoprotective therapy in patients with acute coronary syndrome (myocardial infarction), who underwent balloon angioplasty and coronary artery stenting. Archive of Clinical Medicine.2019;(1):31-7.

DOI: https://doi.org/10.21802/acm.2019.1.2

7. Astashkin EI, Glezer MG. Role of L-carnitine in energy metabolism cardiomyocytes and treatment of diseases of cardiovascular system. Cardiology and cardiovascular surgery. 2012;6(2):58-65.

8. DiNicolantonio JJ, Lavie CJ, Fares H, Menezes AR, O'Keefe JH. L-carnitine in the secondary prevention of cardiovascular disease: systematic review and meta-analysis. In Mayo Clinic Proceedings 2013 Jun 1 (Vol. 88, No. 6, pp. 544-551). Elsevier.

9. Celermajer DS. Endothelial dysfunction: does it matter? Is it reversible?. Journal of the American College of Cardiology. 1997 Aug;30(2):325-33.

DOI: https://doi.org/10.1016/S0735-1097(97)00189-7

10. Colonna P, Iliceto S. Myocardial infarction and left ventricular remodeling: results of the CEDIM trial. American heart journal. 2000 Feb 1;139(2): s124-30.

DOI: https://doi.org/10.1067/mhj.2000.103918

11. Haynes WG, Webb DJ. Endothelin as a regulator of cardiovascular function in health and disease. Journal of Hypertension. 1998 Aug 1;16(8):1081-98.

DOI: https://doi.org/10.1097/00004872-199816080-00001

12. George J, Shmuel SB, Roth A. L-arginine attenuates lymphocyte activation and antioxidized LDL antibody levels in patients undergoing angioplasty. Atherosclerosis. 2004;174: 323-327.

DOI: https://doi.org/10.1016/j.atherosclerosis.2004.01.025

13. Ibanez B, James S, Agewall S. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of

acute myocardial infarction in patients presenting with ST-segment elevation. Eur Heart J. 2018;2: 119-177.
DOI: https://doi.org/10.1093/eurheartj/ehx393.
14. Opie LH. Role of carnitine in fatty acid metabolism of normal and ischemic myocardium. Am Heart J. 1997;97 (3):375-88.
DOI: https://doi.org/10.1016/0002-8703(79) 90440-X
15. Rizzon P, Biasko G, Biase MDi. High doses of L-carnitine in acute myocardial infarction: metabolic and antiarrhythmic effects. Eur Heart J.1989;10 (6):502-8.
DOI: https://doi.org/10.1093/oxfordjournals. eurheartj.a059519
16. Shug A, Thomsen J, Folts J. Changes in tissue levels of carnitine and other metabolites during myocardial ischemia and anoxia. Arch Biochem Biophys. 1998;187(1):25-33.
DOI: https://doi.org/10.1016/0003-9861(78) 90003-6
17. Hicks JJ, MontesCortes DH, Cruz-Dominguez MP. Antioxidants decrease reperfusion induced arrhythmias in myocardial infarction with ST-elevation. Frontiers in Bioscience. 2007;12:2029-37.
DOI: https://doi.org/10.2741/2208

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ELAEOCARPUS SERRATUS L. EXHIBITS POTENTIAL ANALGESIC AND ANTIDIARRHEAL ACTIVITIES IN MICE MODEL

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Background. *Elaeocarpus serratus L. (Family: Elaeocarpaceae) is a tropical fruit tree, traditionally used in the treatments of poisoning, diarrhea, arthritis, and other diseases.*

Objectives. *The current study was performed to conduct the analgesic, antidiarrheal, and hypoglycemic activity of E. serratus in mice model using methanolic bark crude extract.*

Methods. *To assess the peripheral and central analgesic activity, the acetic acid-induced writhing and tail immersion methods were respectively used. The castor-oil mediated antidiarrheal method was used to assess the antidiarrheal activity whereas, the tail tipping technique was conducted to determine the hypoglycemic activity of the plant extract.*

Results. *In the peripheral analgesic assay, the methanolic bark crude extract of E. serratus significantly inhibits the number of writing 69.77% (200 mg/kg) and 73.26% (400 mg/kg) respectively ($p<0.05$) which was strongly comparable with standard NSAID drug diclofenac sodium 75.58% ($p<0.05$). Similarly, it shown a significant tail flicking response for 30 minutes, 60 minutes and 90 minutes of central analgesic activity assay. In antidiarrheal activity assay, the E. serratus substantially reduced the number of diarrheal feces 64.26% (200 mg/kg, $p<0.05$) and 78.57% (400 mg/kg, $p<0.05$) which was also comparable with the positive control loperamide. The hypoglycemic activity of E. serratus extract was not convincing.*

Conclusions. *Our investigation demonstrated the significant analgesic and antidiarrheal activities of methanolic bark extract of E. serratus (200 and 400 mg/kg) in mice model.*

KEYWORDS: ***Elaeocarpus serratus*; analgesic activity; antidiarrheal activity; hypoglycemic activity.**

Introduction

Medicinal plants or natural drugs are traditionally and historically used around the globe by human beings for curing various ailments. The plant-derived natural drugs are widely accepted to all due to their diverse pharmacological activities, reduced toxicity, cost-effective, availability for drug discovery, and application to the chemical biology [1, 2]. Although, the incessant investigation is being carried out to screen potential pharmacological activities of natural products but the numbers are very limited considering all medicinal plants distributed throughout the world [3]. So far, a considerable number of experimental research have been reported the use of natural products as an antioxidant agent, blood glucose-lowering agent, antimicrobial agent, central nervous system (CNS) stimulating agent, anti-diarrheal agent, anti-helminthic agent, anti-inflammatory agent, and anti-cancer agent [4]. By considering the previous studies, we explored the pharmacological activities of *Elaeocarpus serratus* L. *(E. serratus)* in a number of biological uses.

E. serratus (English name: Rosary nut, Ceylon olive, Bengali name: Jalpai) belongs to the family Elaeocarpaceae, a tropical fruit tree grown up to 18 meters tall, distributed in evergreen forests, and sometimes also cultivated for its edible fruit and medicinal applications [5, 6]. It is mostly found in the Indian subcontinent regions including India, Bangladesh, Pakistan, Sri Lanka and Nepal. However, it is also found in Indo-China regions including, Myanmar, Indonesia, Thailand and Malaysia [5]. The *E. serratus* is a plant having both nutritional and medicinal values. For instance, the GC-MS analysis revealed that the plant contains numerous compounds including fatty acid, alcohol, aldehyde, hydrocarbons and alkenes which are biologically active [7]. In

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addition, the leaf of *E. Serratus* contains alkaloids, flavonoids, and glycosides (eg. anthraquinone) [7]. Moreover, a list of bioactive compounds also contained in E. *serratus* such as myricitrin, mearnsetin 3-O-β-D-glucoside, mearnsitrin, and tamarixetin 3-O-α-L-rhamnopyranoside where, myricitrin is an established potential antioxidant [8]. Historically, leaves of *E. serratus* extracts are used for the treatments of arthritis and various poisoning [9]. Equally, appetite, diarrhea, dysentery and other neuro-motors related diseases are commonly treated with fruits or fruit extracts [6, 10]. Moreover, the previous studies also reported that the leaf, bark and fruit of *E. serratus* have antimicrobial and antifungal activities [11, 12]. For all we know, there is no scientific report conducted on analgesic, hypoglycemic, and anti-diarrheal properties of *E. serratus* yet. Therefore, our main objective was to assess the analgesic, hypoglycemic, and antidiarrheal activity of methanolic bark crude extract of *E. serratus* in mice model.

Methods

Collection and extraction of plant

In February 2018, the bark of *E. Serratus* was acquired from Chandpur, Bangladesh. The collection of bark samples was verified by Bangladesh National Herbarium (BDNH), Dhaka, Bangladesh. An herbarium specimen number (DACB-31155) was provided and preserved for their further reference. The barks were cleaned and cut into small pieces to accelerate the drying process. Then the sun-dried fragments were crushed to a fine powder. About 400 g of powder was put in a flat bottom amber sterile glass container and socked with 1.5L methanol for two weeks. Continuous shaking and stirring were maintained over time. Afterward, the entire mixture was filtered with cotton and repeated second filtration with Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was then kept for a week allowed to concentrate with a rotary evaporator at 45°C and 50 rpm. Finally, 28.0 g (yield 5.63%) of a black gummy substance was obtained referring to crude methanol extract of *E. serratus* bark. The extract was aliquot into 2 ml centrifuge tubes and stored at 4°C for further uses.

Experimental animals

To conduct the experiments, the Swiss albino mice (20-24gm) were brought from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR). The animals had a typical environmental condition (at 24.0 ± 1°C and 55 - 65% relative humidity), in cages with 12 hours of dark and light cycles. Until starting of the experiments, the animals were housed to embrace the local laboratory conditions for a week. In each bioassay, the animals were selected randomly and sub-divided into four separated groups consist of positive control (PC) group, negative control (NC) group and two experimental groups receiving *E. serratus* crude extract at doses of 200 mg/kg body weight (b.w.) (ES-I) and 400 mg/kg b.w. (ES-II). Mice were remarkably observed for a week to monitor any suffering or distress and fasted overnight prior to the experiments. The animal experiments were conducted according to the Ethics Committee of State University of Bangladesh (SUB), Dhaka, Bangladesh.

Drug treatments and chemical reagents

Diclofenac sodium, glibenclamide, and loperamide hydrochloride were purchased from Beximco Pharmaceuticals Ltd (Bangladesh). Phenobarbitone sodium and morphine sulphate were supplied by Incepta Pharmaceuticals Ltd (Bangladesh), and Beacon Pharmaceuticals Ltd (Bangladesh). Tween 80, normal saline (0.9% NaCl) and castor oil were kindly given by BDH Chemicals Ltd (United Kingdom). The remaining chemicals and reagents were purchased from Sigma-Aldrich (Munich, Germany).

Peripheral analgesic activity

The acetic acid-mediated writhing method by Kaushik, D., et al. 2012 was copied to assess the peripheral analgesic activity of the *E. serratus* crude extract [13]. The intraperitoneal acetic acid injection was given to all mice with a view to exhort the abdominal pain followed by writhing in mice. The potentialities of the test samples were measured by calculating their competency in the reduction of writhing numbers. Test group (ES-I and ES-II) were orally administered, containing the doses of 200- and 400 mg/kg of body weight (b.w.), respectively. Whereas, the NC group orally received 1% tween 80 in saline and the PC group orally received diclofenac sodium at 50 mg/kg dose [14]. To induce writhing in mice, 1% v/v acetic acid was given intraperitoneally to all mice (10 ml/kg b.w.) followed by a resting period of 40 minutes after test samples administration. The writhing cases were carefully observed and documented for 10 minutes after giving 10 minutes resting period. The acetic acid-induced pain reduction was calculated by using the following equation:

$$\%\ of\ writhing\ inhibition = \frac{(Mean\ writhing\ of\ control - Mean\ writhing\ of\ test) \times 100\%}{Mean\ writhing\ of\ control}$$

Central analgesic activity

Pizziketti et al., 1985 described the tail-flick method was implemented to assess the central analgesic activity of *E. serratus* crude extract in mice [15]. In this method, the mice were orally given a different dose of drugs and *E. serratus*, and the tips of the mice tails were submerged in a constant radiant heat source (hot water bath at 55±0.5 °C). The reaction time (mice tail deflects from the heating source) of each mouse was recorded using a stopwatch. To prevent the damage of tail, we maintained a cut off period of 15 seconds. Similar to the peripheral analgesic study, the NC group orally received 1% tween-80 in saline, and the PC group was subcutaneously injected with morphine (2 mg/kg b.w.) [16]. The ES-I and ES-II were prescribed orally to the test groups of mice. The tail-flick reaction was counted and recorded in 0 minutes, 30 minutes, 60 minutes, and 90 minutes after administration of the test samples. The following equation was used to measure the pain inhibition percentage (PIP).

$$PIP = \frac{(Mean\ latency\ of\ treatment - Mean\ latency\ of\ control) \times 100\%}{Mean\ latency\ of\ control}$$

Hypoglycemic activity

The tail tipping technique according to the method described by Durschlag et al., 1996 was repeated to assess the hypoglycemic activity of test samples in mice model [17]. Here, the NC group was treated with 1% tween-80 (0.1 ml/10 mg b.w.), and PC group treated with glibenclamide (5 mg/kg b.w.) whereas, Group-III and Group-IV were treated with ES-I and ES-II respectively. All treatments were applied orally [14]. To accelerate the blood sugar level of mice, a 10% glucose solution was orally given to all mice after an hour resting period at dose 2 g/kg b.w. Blood is withdrawn from the tail tip and blood sugar was measured and recorded by using a glucometer (Bioland G-423 S) in 0 minutes, 30 minutes, 60 minutes, 120 minutes, and 180 minutes respectively.

Antidiarrheal activity

The antidiarrheal activity of *E. serratus* crude extract was determined by the method described by Shaoba and Thomas [18], where forceful diarrhea is induced by orally administrating 1.0 ml of castor oil to all mice. Similar to other studies, various oral treatments were applied to the mice such as NC group received 1% tween-80 in saline, PC group received loperamide hydrochloride (50 mg/kg b.w.) and the remaining two groups were given ES-I and ES-II respectively [19]. All groups of mice were housed in individual cages with a blotting paper placed beforehand. The number of diarrheal feces were recorded for each mice over four hours of the experiment. The percentage of diarrheal prohibition was accounted for using the following formula:

$$Percentage\ inhibition = \frac{Mean\ defecation\ of\ control - Mean\ defecation\ of\ test\ sample\ or\ standard}{Mean\ writhing\ of\ control} \times 100\%$$

Statistical analysis

The values are represented here are set of mean ± standard error of mean (M±SEM). All the calculation was performed using student t-test or one way ANOVA followed by Dunnett's test to determine the statistically significant differences between the groups. A p-value < 0.05 was considered statistically significant.

Results

The peripheral analgesic activity of *E. serratus* crude extract is demonstrated in Table 1. A significant reductions of abdominal muscle contractions caused by the administration of 0.1 ml acetic acid were exhibited in both experimental groups where ES-II showed higher writhing inhibition and was close to the PC group. Our results indicated that the *E. serratus* bark crude extracts significantly inhibit the number of writhing 69.77% and 73.26% at dose 200 and 400 mg/kg b.w. gradually whilst diclofenac sodium displayed 75.58% writhing inhibition.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC= diclofenac sodium, ES-I: *E. serratus* crude extract-I, ES-II: *E. serratus* crude extract-II. M1-4=mice 1 to 4 respectively. (n=4, *p<0.01)

The result of the tail-flick method to assess the central analgesic activity of *E. serratus* are

Table 1. Peripheral analgesic activity of *E. serratus* bark crude extract

Mice group	Writhing count (sec)				Number of writhing (Mean±SEM)	% Inhibition of writhing
	M-1	M-2 M-3	M-3	M-4		
NC	20	21	23	22	21.50±0.65*	–
PC	5	6	5	5	5.25±0.25*	75.58
ES-I	7	6	6	7	6.50±0.29*	69.77
ES-II	6	6	5	6	5.75±0.25*	73.26

shown in table 2. Both experimental groups ES-I and ES-II increased the response time by 37.32% and 53.72% respectively in the initial 30 minutes of the experiment, whereas PC morphine increased by 85.24%. In addition, 82.76% (200 mg/kg b.w.), 98.94% (400 mg/kg b.w.) tail flicking response were recorded in 60 minute, and 149.27% (200 mg/kg b.w.) and 179.46% (400 mg/kg b.w.) were recorded in 90 minutes of the experiments. The whole experiment followed a dose-dependent tail flicking response over the time.

Values are represented here as mean of ±SEM. NC= 1% tween 80 in water, PC= morphine sulfate, ES-I: *E. serratus* crude extract-I, and ES-II: *E. serratus* crude extract-II. (n=4, *p<0.01)

The bark crude extract of *E. serratus* not displayed any significant blood glucose-lowering activity at doses 200 and 400-mg/kg b.w. However, the percent of blood sugar reducing activity was found to be followed in a dose-dependent manner. The results shown in table 3 indicated that the highest glucose lowering activity was displayed at dose 400 mg/kg b.w. relative to ES-I groups.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC=glibenclamide, ES-I: *E. serratus* crude extract-I, and ES-II: *E. serratus* crude extract-II. (n=4, *p<0.01)

The remarkable antidiarrheal activities were displayed by ES-I and ES-II in mice. The potential antidiarrheal activity of the *E. serratus* crude extract is shown in table 4. The ES-I and ES-II substantially reduced the number of castor oil-incited diarrheal feces by 64.29% and 78.57% compared to the NC. The highest diarrheal reduction was shown by PC group.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC=loperamide hydrochloride, ES-I: *E. serratus* crude extract-I,

Table 2. Central analgesic activity of *E. serratus* bark crude extract

Mice group	30 minutes of assay		60 minutes of assay		90 minutes of assay	
	M±SEM	% of elongation	M±SEM	% of elongation	M±SEM	% of elongation
NC	3.49±0.32*	–	3.55±0.08*	–	3.60±0.20*	–
PC	6.47±0.11*	85.24	9.57±0.25*	169.39	13.19±0.35*	266.07
ES-I	4.79±0.34*	37.32	6.49±0.35*	82.76	8.98±0.25*	149.27
ES-II	5.37±0.32*	53.72	7.07±0.39*	98.94	10.07±0.42*	179.46

Table 3. Hypoglycemic activity of *E. serratus* bark crude extract

Mice group	60 minutes of assay		120 minutes of assay		180 minutes of assay	
	M±SEM (mmol/L)	% Reduction	M±SEM (mmol/L)	% Reduction	M±SEM (mmol/L)	% Reduction
NC	12.60±0.87		8.55±0.31		4.45±0.34	
PC	5.63±0.39	55.36	4.23±0.57	50.58	2.6±0.17	41.57
ES-I	10.18±0.61	19.25	7.05±0.39	17.54	3.95±0.23	11.24
ES-II	10.08±0.93	20.04	6.85±0.38	19.88	3.75±0.19	15.73

Table 4. Antidiarrhoeal activity of *E. serratus* bark crude extract

Mice group	Dose	Number of diarrheal feces (Mean±SEM)	% Reduction of diarrhea
NC	10 ml/kg b.w.	3.5±0.58*	–
PC	50 mg/kg b.w.	0.5±0.48*	85.71
ES-I	200 mg/kg b.w.	1.25±0.85*	64.29
ES-II	400 mg/kg b.w.	0.75±0.71*	78.57

and ES-II: *E. serratus* crude extract-II. (n=4, *p<0.01)

Discussion

Acetic acid may trigger the writhing reflex in experimental animals where visceral pain is generated through activation of pain receptors on the visceral surface and extreme secretion of histamine, prostaglandins, bradykinin and serotonin [20]. In the experimental animals, acetic acid induces visceral pain which is commonly treated with NSAID drugs or chemicals, such as phenyl quine (prostaglandin E2 inhibitor). In addition, the level of analgesia is measured by calculating the percent reduction of abdominal contraction by drugs or crude extract after intraperitoneal administration of acetic acid to mice. In this study, *E. serratus* extracts significantly reduced the sum of abdominal contraction of 69.77% and 73.26% by ES-I and ES-II compared to NC. Importantly, the results of peripheral analgesic activity by ES-I and ES-II were almost equal to the diclofenac treatment. Therefore, by considering our results, we assumed that *E. serratus* extract may be inhibited the synthesis or release of endogenous substances in mice to act its potential peripheral analgesic activity. However, further research may need to explore the exact mechanisms.

In the central analgesic assay, the relative promotion of tail-flicking response (in percent) was obtained from *E. serratus* extract in a dose and time-dependent manner. Although, the responses from *E. serratus* crude extracts were a bit of lower than the PC-morphine however, higher dose might be shown an equals or higher potentiality like morphine. Pizziketti, et al., 1985 demonstrated that the tail flicking response is mostly generated from spinal reflex caused by radiant heat source however it may involve higher neuronal complex signals. In general, the pain is centrally originated via a number of complex signaling such as opiate, dopaminergic, noradrenergic and serotonergic nervous systems [15]. Our results described that *E. serratus* displayed a significantly higher level of pain threshold activity at 200 and 400 mg/kg b.w. respectively in mice model. The core mechanisms may be associated with the receptor-bind inhibition of pain-related nervous system or through peripheral mechanisms involved prohibited prostaglandins, leukotrienes, and other endogenous substances release and synthesis which are key mediators of pain [21]. Our results might be followed the same mechanisms to exhibit the potential analgesic activity in mice model.

Our bark crude extract of *E. serratus* shown lack of blood glucose lowering activity. Notwithstanding, a considerable number of studies have concluded that plant extracts exhibit potential anti-hyperglycemic activity by accelerating or regenerating β cells or promoting the secretion of insulin [22, 23]. The hypoglycemic activity by the natural product may also associated with excessive insulin secretion from β cells or trigger the peripheral glucose consumption, or promote insulin-mediated blood sugar absorbing mechanisms [22-24].

Apart from this, the statistical evaluation revealed that both doses of *E. serratus* showed a significant dose-dependent anti-diarrheal activity in mice. The ricinoleic fatty acid or 12-hydroxy-9-cis-octadecenoic acid is an active metabolite of castor oil. This metabolic fatty acid enhanced peristaltic activity in the small intestine to trigger the permeability of mucosal electrolytes thus resulting diarrhea [25, 26]. Furthermore, ricinoleic fatty acid enhanced mucosal irritation and inflammation which contribute to the excessive endogenous prostaglandin secretion. Moreover, in castor oil-induced diarrheal mechanisms it involved a cascade of signaling including, intestinal Na^+/K^+-ATPase inhibition, adenylate cyclase activation or promotion cAMP-mediated platelet-activating factor secretion [25, 27].

In summary, the plant *E. serratus* contained several flavonoids, anthraquinone glycosides, fatty acid, alcohol, aldehyde, hydrocarbons alkaloids, terpenoids, and steroids. [7, 8, 28]. The presence of glycosides, steroids, and flavonoids which exhibited potential analgesic, hypoglycemic and antidiarrheal activities in many plants [29-31]. In the present study, we concluded that *E. serratus* extract may contain a variety of bioactive phytochemicals. After successful isolation and characterization of phytochemicals, it might be used as an analgesic, and as an antidiarrheal agent.

Conclusion

The bark extract of *E. serratus* exhibited potential peripheral and central analgesic activity, very mild hypoglycemic activity but effective antidiarrhoeal activity in mice model. Therefore, further investigations are needed to isolate and char*a*cterization of bioactivite molecules present in this plant. Further research may open a new therapeutic agents in the treatments of various diseases.

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Conflict of Interests

Authors declare no conflict of interest.

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Author's Contributions

Asma Aul Husna Pinkey, Mohammad Abdullah Taher – conceptualization, methodology; *Asma Aul Husna Pinkey,* investigation, data curation, formal analysis, writing – original draft; *Zahirul Islam Khan* – data curation, formal analysis, writing – reviewing and editing; *Mahfuza Afroz Soma* – writing – reviewing and editing.

ПРЕКЛІНІЧНІ ДОСЛІДЖЕННЯ ЗНЕБОЛЮЮЧОЇ ТА ПРОТИПРОНОСНОЇ ДІЇ *ELAEOCARPUS SERRATUS L.* НА МИШАХ

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Вступ. *Elaeocarpus serratus L. (родина Elaeocarpaceae) - тропічне плодове дерево, фрукти, кора та інші частини якого традиційно використовуються при лікуванні отруєнь, діареї, артриту та інших захворювань.*

Мета – *дослідити фармакологічну активність (знеболювальну, протидіарейну та гіпоглікемічну дію) сухого метанольного екстракту кори E. serratus на мишах.*

Методи. *Для експериментальної оцінки центрального та периферичного компонентів у механізмі знеболювальної дії екстракту використовували метод оцінки больової реакції, що викликається хімічним подразненням – метод «оцтовокислих корчів», та метод теплового подразнення, суть якого полягає в зануренні хвоста миші у гарячу воду (55±0.5°C). Для оцінки протипроносної активності використовували модель діареї, викликаної введенням рициновою олією, для визначення гіпоглікемічної активності екстракту використали метод Durschlag et al., 1996, забір крові проводили шляхом надрізів хвоста.*

Результати. *Встановлено, що застосування сухого метанольного екстракту кори E. serratus достовірно зменшує частоту розвитку корчів на 69,77% (200 мг/кг) та 73,26% (400 мг/кг) відповідно ($p<0,05$), що досягає рівня активності стандартного НПЗП диклофенаку натрію, який зменшує показник на 75,58% ($p<0.05$). Такі ж результати щодо частоти реакції хвоста піддослідних тварин протягом 30, 60 та 90 хвилин – показника центральної знеболюючої активності екстракту. Щодо протипроносної активності, то E. Serratus зменшував частоту діареї на 64.26% (200 мг/кг, $p<0,05$) та 78,57% (400 мг/кг, $p<0,05$), що також досягало також ж ефективності, які і у групі позитивного контролю з лоперамідом. Щодо гіпоглікемічної активності екстракту E. serratus – отримані нами результати були непереконливими.*

Висновок. *Наше дослідження продемонструвало значну знеболювальну та протидіарейну активність сухого метанольного екстракту кори E. serratus (200 та 400 мг/кг) на мишах.*

КЛЮЧОВІ СЛОВА: ***Elaeocarpus serratus*; знеболювальна активність; протипроносна активність; гіпоглікемічна активність**

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References

1. Saleh-e-In MM, Sultana N, Hossain MN, Hasan S, Islam MR. Pharmacological effects of the phytochemicals of Anethum sowa L. root extracts. BMC Complement Altern Med. 2016 Dec;16(1):464.
DOI: https://doi.org/10.1186/s12906-016-1438-9

2. Khan MF, Khan ZI, Uddin MR, Rahman MS, Rashid MA. In vivo hypoglycemic and alloxan induced antidiabetic activity of Xeromphis uliginosa Retz. Afr J Pharm Pharmacol. 2015;9(11):363-6.
DOI: https://doi.org/10.5897/AJPP2015.4293

3. Al-Snafi AE. Encyclopedia of the constituents and pharmacological effects of Iraqi medicinal plants. Rigi Publication; 2015.

4. Van Wyk B-E, Wink M. Medicinal plants of the world. CABI; 2018.
DOI: https://doi.org/10.1079/9781786393258.0000

5. Baruah PS, Deka K, Lahkar L, Sarma B, Borthakur SK, Tanti B. Habitat distribution modelling and reinforcement of Elaeocarpus serratus L. - A threatened tree species of Assam, India for improvement of its conservation status. Acta Ecologica Sinica. 2019;39(1):42-9.
DOI: https://doi.org/10.1016/j.chnaes.2018.06.002

6. de Lima FF, Breda CA, Cardoso CAL, Duarte MCT, Sanjinez-Argandoña EJ. Evaluation of nutritional composition, bioactive compounds and antimicrobial activity of Elaeocarpus serratus fruit extract. Afr J Food Sci. 2019;13(1):30-7.
DOI: https://doi.org/10.5897/AJFS2018.1760

7. Geetha D, Rajeswari M, Jayashree I. Chemical profiling of Elaeocarpus serratus L. by GC-MS. Asian Pac J Trop Biomed. 2013;3(12):985-7.
DOI: https://doi.org/10.1016/S2221-1691(13)60190-2

8. Jayasinghe L, Amarasinghe NR, Arundathie BS, Rupasinghe GK, Jayatilake NAN, Fujimoto Y. Antioxidant flavonol glycosides from Elaeocarpus serratus and Filicium decipiens. Nat Prod Res. 2012;26(8):717-21.
DOI: https://doi.org/10.1080/14786419.2010.551514

9. Geetha D, Jayashree I, Rajeswari M. In vitro anti-arthritic activity of Elaeocarpus serratus Linn. (Elaeocarpaceae). Int J Pharm Sci Res. 2015;6(6):2649.

10. Hardainiyan S, Nandy BC, Kumar K. Elaeocarpus ganitrus (Rudraksha): A reservoir plant with their pharmacological effects. Int J Pharm Sci Rev Res. 2015;34:55-64.

11. Jayashree I, Geetha D, Rajeswari M. Evaluation of antimicrobial potential of Elaeocarpus serratus L. Int J Pharm Sci Res. 2014;5(8):3467-72.

12. Sneha S, Sharath R, Aishwarya K, Samrat K, Vasundhara M, Radhika B. Screening of the antioxidant, antibacterial and cytotoxic activities of the methanolic extracts of Elaeocarpus ganitrus and Elaeocarpus serratus. Int Res J Innov Eng. 2015;1:1-11.

13. Kaushik D, Kumar A, Kaushik P, Rana AC. Analgesic and anti-inflammatory activity of Pinus roxburghii Sarg. Adv Pharmacol Sci. 2012;2012:245431. doi: 10.1155/2012/245431.
DOI: https://doi.org/10.1155/2012/245431

14. Sharmin T, Rahman MS, Mohammadi H. Investigation of biological activities of the flowers of Lagerstroemia speciosa, the Jarul flower of Bangladesh. BMC Complement Altern Med. 2018;18(1):231.
DOI: https://doi.org/10.1186/s12906-018-2286-6

15. Pizziketti R, Pressman N, Geller E, Cowan A, Adler M. Rat cold water tail-flick: a novel analgesic test that distinguishes opioid agonists from mixed agonist-antagonists. Eur J Pharmacol. 1985;119(1-2):23-9.
DOI: https://doi.org/10.1016/0014-2999(85)90317-6

16. Adeyemi OO, Okpo SO, Ogunti OO. Analgesic and anti-inflammatory effects of the aqueous extract of leaves of Persea americana Mill (Lauraceae). Fitoterapia. 2002;73(5):375-80.
DOI: https://doi.org/10.1016/S0367-326X(02)00118-1

17. Dürschlag M, Würbel H, Stauffacher M, von Holst D. Repeated blood collection in the laboratory mouse by tail incision-modification of an old technique. Physiol Behav. 1996;60(6):1565-8.
DOI: https://doi.org/10.1016/S0031-9384(96)00307-1

18. Shoba FG, Thomas M. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. J Ethnopharmacol. 2001;76(1):73-6.
DOI: https://doi.org/10.1016/S0378-8741(00)00379-2

19. Pal A, Al Mahmud Z, Akter N, Islam S, Bachar SC. Evaluation of Antinociceptive, Antidiarrheal and Antimicrobial Activities of Leaf Extracts of Clerodendrum indicum. Phcog J. 2012;4(30):41-6.
DOI: https://doi.org/10.5530/pj.2012.30.8

20. Padi SSV, Kulkarni SK. Minocycline prevents the development of neuropathic pain, but not acute pain: Possible anti-inflammatory and antioxidant mechanisms. Eur J Pharmacol. 2008;601(1):79-87.
DOI: https://doi.org/10.1016/j.ejphar.2008.10.018

21. Shojaii A, Motaghinejad M, Norouzi S, Motevalian M. Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of Astragalus hamosus in animal models. Iran J Pharm Res. 2015;14(1):263-9.

22. Hannan J, Marenah L, Ali L, Rokeya B, Flatt P, Abdel-Wahab Y. Ocimum sanctum leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic β-cells. J Endocrinol. 2006;189(1):127-36.
DOI: https://doi.org/10.1677/joe.1.06615

23. Gray AM, Flatt PR. Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant Coriandrum sativum (coriander). Br J Nutr. 1999;81(3):203-9.
DOI: https://doi.org/10.1017/S0007114599000392

24. Ota A, Ulrih NP. An overview of herbal products and secondary metabolites used for management of type two diabetes. Front Pharmacol. 2017;8:436.
DOI: https://doi.org/10.3389/fphar.2017.00436
25. Iwao I, Terada Y. On the mechanism of diarrhea due to castor oil. Jpn J Pharmacol. 1962; 12(2):137-45.
DOI: https://doi.org/10.1254/jjp.12.137
26. Bright-Asare P, Binder HJ. Stimulation of colonic secretion of water and electrolytes by hydroxy fatty acids. Gastroenterology. 1973;64(1):81-8.
DOI: https://doi.org/10.1016/S0016-5085(73)80094-0
27. Tiruppathi C, Balasubramanian K, Hill P, Mathan V. Faecal free fatty acids in tropical sprue and their possible role in the production of diarrhoea by inhibition of ATPases. Gut. 1983;24(4):300-5.
DOI: https://doi.org/10.1136/gut.24.4.300
28. Jayashree I, Geetha D, Rajeswari M. Evaluation of antimicrobial potential of Elaeocarpus serratus L. Int J Pharm Sci and Res. 2014;5(8):3467.
29. Zhang D-W, Cheng Y, Wang N-L, Zhang J-C, Yang M-S, Yao X-S. Effects of total flavonoids and flavonol glycosides from Epimedium koreanum Nakai on the proliferation and differentiation of primary osteoblasts. Phytomedicine. 2008;15(1-2):55-61.
DOI: https://doi.org/10.1016/j.phymed.2007.04.002
30. Kajaria DK, Gangwar M, Sharma AK, Nath G, Tripathi Y, Tripathi J, et al. Comparative evaluation of phenol and flavonoid content of polyherbal drugs. Pharmacologyonline. 2011;3:1365-73.
31. Arif M, Fareed S. Pharmacognostical studies and evaluation of total phenolic and flavonoid contents of traditionally utilized fruits of Solanum torvum Sw. Indian J Nat Prod Resour. 2011;2(2):218-24.

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QUALITY CONTROL MEASUREMENT AND *IN VITRO* BIOEQUIVALENCE OF VALSARTAN AND ATENOLOL TABLETS MARKETED IN UKRAINE

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Background. *The urgent issue of hypertension is determined by its high population incidence, significant burden of the disease, risk of disability and impact on life expectancy. Rational combinations of drugs of different pharmacological groups in case of ineffectiveness of monotherapy to achieve the clinical effect of pharmacotherapy are clearly recommended in the world and national recommendations for diagnosis and treatment of hypertension. Therefore, innovative pharmaceutical development of a combination of antihypertensive drugs and creation of domestic drugs with antihypertensive action is an urgent task of contemporary pharmacy.*

Objective. *The aim of this study was to perform the quality control measurements and evaluation of dissolution tests for different brands of valsartan and atenolol tablets available in Ukraine.*

Methods. *The concentrations of valsartan and atenolol in samples (drug content and dissolution study) were determined by the proposed HPLC method.*

Results. *The results of the tests conducted for evaluation of the tablets were found to be in acceptable limits for all the selected brands. The correlation coefficient (R^2) was 0.9991 and the regression equation was y=61.39x+0.3117. It has been established that the equivalence of dissolution profiles for all recommended dissolution media is observed (pH 1.2, 4.5, and 6.8) for the studied drugs. In all three dissolution media, the release rates of valsartan and atenolol of all dosage forms are more than 85% in 15 min. The dissolution profile of all the selected brands was within the standard limits and was acceptable.*

Conclusions. *Analytical method development is an integral part of the quality control measurements and evaluation of dissolution tests. Our previously developed HPLC method is essential for quality control of a large number of samples in short time intervals. Therefore, the method developed by our group is suitable for a routine quality control analysis of any pharmaceutical preparation containing two tested drugs with the suggested chromatographic method advantages for checking quality during dissolution studies of their dosage forms.*

KEYWORDS: **hypertension; valsartan; atenolol; high-performance liquid chromatography; *in vitro* bioequivalence; dissolution.**

Introduction

Valsartan (Fig. 1) is chemically described as (2*S*)-3-methyl-2-[pentanoyl-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl]methyl]amino]butanoic acid. Valsartan is an orally active nonpeptide triazole-derived antagonist of angiotensin (AT) II with antihypertensive properties. Valsartan selectively and competitively blocks the binding of angiotensin II to the AT1 subtype receptor in vascular smooth muscle and the adrenal gland, preventing AT II-mediated vasoconstriction, aldosterone synthesis and secretion, and renal reabsorption of sodium, and results in vasodilation, increased excretion of sodium and water, reduction of plasma volume, and reduction of blood pressure. Therefore, analytical methods for their separation and quantification in pharmaceutical formulations and inhuman plasma are needed for quality control and therapeutic drug monitoring, respectively. Several techniques have been reported in the literature for determination of valsartan individually and combination with other drug other than atenolol [1-16] in pharmaceutical dosage forms or human serum samples.

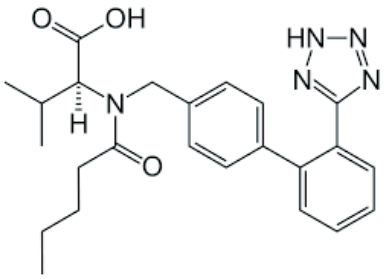

Fig. 1. Chemical structure of valsartan.

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Atenolol (Fig. 2) is a synthetic isopropylamino-propanol derivative used as an antihypertensive, hypotensive and antiarrhythmic. Atenolol is chemically known as 2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide. Atenolol acts as a peripheral, cardioselective beta blocker specific for beta-1 adrenergic receptors, without intrinsic sympathomimetic effects. It reduces exercise heart rates and delays atrio-ventricular conduction, with overall oxygen requirements decrease. Numerous analytical methods were reported [17-27] for determination of atenolol in bulk and combination with other drugs other than valsartan.

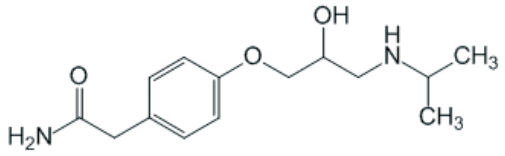

Fig. 2. Chemical structure of atenolol.

The urgent issue of hypertension is determined by its high population incidence, significant burden of the disease, risk of disability and impact on life expectancy. Rational combinations of drugs of different pharmacological groups in case of ineffectiveness of monotherapy to achieve the clinical effect of pharmacotherapy are clearly recommended in the world and national recommendations for diagnosis and treatment of hypertension. Therefore, innovative pharmaceutical development of a combination of antihypertensive drugs and creation of domestic drugs with antihypertensive action is an urgent task of contemporary pharmacy. In the last few decades, the cost of medications is increasing a lot and it is quite challenging to afford lifelong medications for hypertension treatment. Different strategies have been planned by the healthcare systems to reduce the medication costs, improve treatment efficacy and safety and patient compliance with pharmacotherapy [28]. The results of quality control testing, such as friability, weight variation, hardness, percentage purity, and disintegration, suggests the level up to which the GMP guidelines had been followed during the manufacturing of these generic products. When the generic and the innovator brand would have comparable dissolution profile then the *in vivo* bioequivalence test of the generics can be waived. It was reported that fewer generics in the market were counterfeit and of inferior quality than the innovators. So, identifying these fake and suspicious generics is the prime challenge to our health department and quality control units. This research assists highlighting the pharmaceutical products which are found to be spurious, of inferior quality and dangerous to the users.

The objective of the research was to perform the quality control measurements and evaluate dissolution tests of different brands of valsartan and atenolol tablets available in Ukraine.

Methods

Valsartan (purity 99.9%) was purchased from Jubilant Generics Limited (India); atenolol (purity 98.9%) was purchased from Sigma-Aldrich (Switzerland). The methanol and acetonitrile used in experiments was HPLC gradient grade and ammonium acetate and tetramethylammonium hydroxide were of Ph.Eur.reagent grade and was purchased from Merck Darmstdat, Germany. Analytical Balance Mettler Toledo MPC227, pH–metter Metrohm 827, demineralized water by TKA Micro system, with final conductivity less than 0.05μS/cm, were used. IKA orbital shaker KS4000i was used for sample agitation. The nylon and regenerated cellulose RC 0.45um syringe filters were purchased from Agilent Technologies.

Dionex Ultimate 3000 UHPLC system controlled by Chromeleon version 6,80, composed of quaternary LPG pump ultimate 3000, autosampler ultimate 3000, ultimate 3000 column compartment, four channel UV-Vis detector ultimate 3000 RS. Shimadzu Nexera XR UPLC system with LPG Quaternary Pump LC-20AD with degasser DGU-20A5R, Autosempler SIL-20AC, PDA detector M20-A, Column Oven and Controller CBM-20A controlled by Lab Solutions version 5,97. The column Discovery C18 (4.6 mm i.d.×150 mm, 5 μm), purchased from Sigma-Aldrich Supelco, was used.

Sample preparation

Twelve tablets of each preparation were studied to obtain statistically significant results. The tablets of different pharmaceutical manufacters with declared contents of 80 mg valsartan and 100 mg of atenolol were purchased from local drug store, pharmacy. The tablets were put in 100 mL measuring flasks and dissolved in 50 mL 50% v/v methanol, ultrasound crushed and treated for 2 minutes and shake 15 minutes with orbital shaker. After that measuring flasks were filled to mark of 100 mL, the final concentrations were 1mg/mL for atenolol and 0.8 mg/mL for valsartan. The samples

were filtered with RC 0.45um syringe filters and injected.

In vitro dissolution of twelve tablets containing valsartan and atenolol was performed using buffer solutions (pH 1.2; 4.5; 6.8) as the dissolution media at 50 rpm by the USP Apparatus II. The dissolution was studied in a 900 mL volume of buffer solution at 37 °C (±0.5) using the paddle method. One mL of sample was withdrawn and replaced with fresh dissolution medium at the time intervals of 5, 15, 30, 45 minutes [29-32].

The concentrations of valsartan and atenolol in the samples (drug content and dissolution investigation) were determined by the suggested HPLC method.

Results

Previously, we have made method development of valsartan and atenolol in dosage forms. The optimum mobile phase composition was composed of 20% acetonitrile, 80% of 0.16% ammonium acetate and 0.2% of 1.5 M tetramethylammonium hydroxide (V/V), pumped with 1.0 mL/min at 30 °C set temperature of column oven, with UV detector set to 225 nm and 237 nm wavelength. Analyses were performed by means of the column Discovery C18 (4.6 mm i.d.×150 mm, 5 µm) (Fig. 3).

The results of percentage purity of all the brands are shown in Table 1. The drug content was determined to be highest for Valsartan-1 and Atenolol-4. The drug content was assessed once and compared with the calibration curve. The correlation coefficient (R^2) was 0.9991 and the regression equation was y = 61.39x+0.3117.

Table 1. Drug content of different brands of valsartan and atenolol tablets

Brand code	Drug content (%) n=20
Valsartan-1 (Innovator)	99.82±2.24
Valsartan-2	98.12±3.65
Valsartan-3	99.11±2.74
Valsartan-4	98.01±3.64
Atenolol-1 (Innovator)	99.11±1.89
Atenolol-2	97.62±3.67
Atenolol-3	98.55±1.95
Atenolol-4	99.92±2.73

Dissolution test is used to determine the quality of the drug. Comparative dissolution kinetics test is used at all stages of the drugs life cycle. In the development of dosage form for comparative dissolution kinetics test allows assessing the technological correctness of techniques, and thereby increase the probability of positive results for future studies of bioequivalence. In addition to routine quality control tests, comparative dissolution tests are used to waive bioequivalence requirements (biowaivers) for lower strengths of a dosage form. Dissolution study is an important parameter used to predict the bioavailability and *in vivo* drug release performance. Dissolution study is very significant in determining the release of drug from different dosage forms including tablets. The active absorption of oral dosage forms depend on adequate release of drug. Comparative dissolution profiles are shown in Table 2.

The point of 15 min is critical and decisive. Medicine is considered very quick soluble when

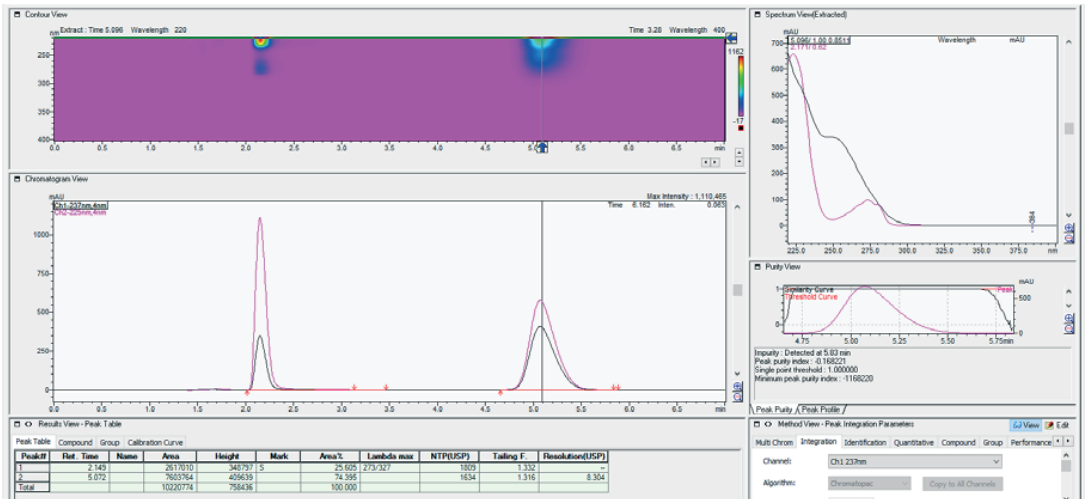

Fig. 3. Chromatogram obtained using Shimadzu Nexera XR UPLC system and mobile phase 20% acetonitrile, 80% of 0.16% ammonium acetate and 0.2% of 1.5 M tetramethylammonium hydroxide (V/V), column Discovery C18 (4.6 mm i.d.×150 mm, 5 µm) at 2 wavelengths 225 nm and 237 nm.

Table 2. Comparative dissolution data of valsartan and atenolol in selected brands

Brand code	Medium	% dissolved 15 min	% dissolved 30 min
Valsartan-1	pH 1.2	95.18	94.59
	pH 4.5	93.84	93.02
	pH 6.8	92.43	93.67
Valsartan-2	pH 1.2	89.98	87.37
	pH 4.5	87.38	89.84
	pH 6.8	85.19	86.38
Valsartan-3	pH 1.2	85.28	86.58
	pH 4.5	87.68	90.01
	pH 6.8	86.84	88.67
Valsartan-4	pH 1.2	85.89	85.98
	pH 4.5	85.96	85.78
	pH 6.8	86.94	86.05
Atenolol-1	pH 1.2	93.96	95.95
	pH 4.5	92.56	93.36
	pH 6.8	90.93	92.58
Atenolol-2	pH 1.2	91.94	92.67
	pH 4.5	89.49	93.06
	pH 6.8	87.56	90.94
Atenolol-3	pH 1.2	91.82	93.37
	pH 4.5	90.01	91.65
	pH 6.8	86.05	89.95
Atenolol-4	pH 1.2	95.96	96.03
	pH 4.5	92.94	94.56
	pH 6.8	91.46	93.14

at least 85% of the active substance dissolves in 15 minutes, quickly soluble – when at least 85% of the active substance dissolves in 30 minutes. According to the obtained data, the equivalence of dissolution profiles for all recommended dissolution media has been established (pH 1.2, 4.5, and 6.8) for the studied drugs. In all three dissolution media, the releases of valsartan and atenolol of all dosage forms were more than 85% in 15 min (Table 2). The dissolution profile of all the selected brands was within the standard limits and was acceptable.

Conclusions

Analytical method development is an integral part of the quality control measurements and evaluation of dissolution test. Our previously developed HPLC method was essential for quality control of a large number of samples in short time intervals. Unavailability and price of innovator brand urges patients to go for alternate options including generic brands. The selected brands were evaluated and compared with those of reference or innovator brand to assure the potential for cure of the disease. Moreover, the results of dissolution studies of all the brands were within the standard limits. This suggested that the proper GMP guidelines were followed during the manufacturing of these brands that proved a good quality. Hence, these generics may be considered to be a substitute for innovator brand in case of unavailability. Thus, all the brands selected for the study complied with the standard specifications and the definite observations on similar efficacy of these generics may be obtained after performing the *in vivo* studies. Therefore, the method developed by our group is suitable for the routine quality control analysis of any pharmaceutical preparation containing two tested drugs with the suggested chromatographic method with advantages for checking quality during dissolution studies of their pharmaceutical preparations.

Conflict of Interests

Authors declare no conflict of interest.

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ВИЗНАЧЕННЯ КОНТРОЛЮ ЯКОСТІ ТА *IN VITRO* БІОЕКВІВАЛЕНТНОСТІ ТАБЛЕТОК ВАЛСАРТАНУ ТА АТЕНОЛОЛУ РИНКУ УКРАЇНИ

К. Пелешок
ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Актуальність проблеми артеріальної гіпертензії визначається її високою популяційною частотою, значним тягарем хвороби, ризиком інвалідизації та впливом на тривалість життя людини. У світових і вітчизняних рекомендаціях з діагностики та лікування артеріальної гіпертензії чітко рекомендовано раціональні комбінації препаратів різних фармакологічних груп при неефективності монотерапії для досягнення клінічного ефекту фармакотерапії. Тому, інноваційна фармацевтична розробка комбінації антигіпертензивних засобів та створення вітчизняних лікарських засобів антигіпертензивної дії є актуальним завданням сучасної фармації.*

Мета. *Здійснити контроль якості та оцінити тест розчинення різних марок таблеток валсартану та атенололу, які є представленими на ринку України.*

Методи. *Концентрації валсартану та атенололу у зразках (вміст в лікарських формах та тест розчинення) визначалися запропонованим методом ВЕРХ.*

Результати. *Результати випробувань, проведених для кількісного визначення визначення АФІ таблеток, є прийнятними для всіх обраних марок. Було встановлено, що коефіцієнт кореляції (R^2) становить 0.9991 та рівняння регресії y=61.39x+0.3117. Доведено, що для досліджуваних препаратів спостерігається еквівалентність профілів розчинення для всіх рекомендованих середовищ розчинення (pH 1.2, 4.5 та 6.8). У всіх трьох середовищах розчинення вивільнення валсартану та атенололу всіх лікарських форм перевищують 85% за 15 хв. Оцінка розчинення всіх вибраних марок в межах стандартних лімітів та є прийнятною.*

Висновки. *Розробка аналітичної методики є невід'ємною частиною контролю якості та оцінки тесту розчинення. Розроблена нами методика ВЕРХ є важливою для контролю якості великої кількості зразків за короткі проміжки часу. Тому метод розроблений нашою групою, підходить для рутинного аналізу якості будь-якого лікарського препарату, що містить два випробувані АФІ із запропонованими перевагами хроматографічного методу для перевірки якості під час досліджень розчинення їх лікарських форм.*

КЛЮЧОВІ СЛОВА: **гіпертензія; валсартан; атенолол; високо ефективна рідинна хроматографія; *in vitro* біоеквівалентність; розчинення**

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References

1. Gupta KR, Wadodkar AR, Wadodkar SG. UV-Spectrophotometric methods for estimation of Valsartan in bulk and tablet dosage form. International Journal of ChemTech Research. 2010;2:985-89.

2. Rao GS, Rao GV, Vardhan S, Ramachandran D. Development and validation of new UV-spectrophotometric assay method for valsartan in pure and in formulations. Journal of Chemical and Pharmaceutical Research. 2013;5:229-32.

3. Ramachandran S, Mandal BK, Navalgund SG. Simultaneous spectrophotometric determination of valsartan and ezetimibe in pharmaceuticals. Tropical Journal of Pharmaceutical Research 2011;10:809-15. DOI: https://doi.org/10.4314/tjpr.v10i6.15

4. Nikam MB, Dhamane H, Aligave A, Kondawar MS. Simultaneous estimation of valsartan, amlodipine besylate and hydrochlorothiazide by first order derivative UV spectrophotometric method. Int J Pharm Technol. 2010;2:642-50.

5. Selvan PS, Gowda KV, Mandal U, Solomon WDS, Pal TK. Simultaneous determination of fixed dose combination of nebivolol and valsartan in human plasma by liquid chromatographic-tandem mass

spectrometry and its application to pharmacokinetic study. Journal of Chromatography B. 2007;858:143-50.
DOI: https://doi.org/10.1016/j.jchromb.2007.08.016
6. Sabi-mouka EMB, Agbokponto JE, Zhang R, Li Q, Ding L. Simultaneous determination of a fixeddose combination of lercanidipine and valsartan in human plasma by LC–MS-MS: Application to a pharmacokinetic study. Journal of chromatographic science. 2016;54:1553-59.
DOI: https://doi.org/10.1093/chromsci/bmw102
7. Koseki N, Kawashita H, Hara H, Niina M, Tanaka M, Kawai R, Nagae Y, Masuda N. Development and validation of a method for quantitative determination of valsartan in human plasma by liquid chromatography-tandem mass spectrometry. Journal of pharmaceutical and biomedical analysis. 2007;43:1769-74.
DOI: https://doi.org/10.1016/j.jpba.2006.12.030
8. Tian DF, Tian XL, Tian T, Wang ZY, Mo FK. Simultaneous determination of valsartan and hydrochlorothiazide in tablets by RP-HPLC. Indian journal of pharmaceutical sciences. 2008;70:372.
DOI: https://doi.org/10.4103/0250-474X.43006
9. Chitlange SS, Bagri K, Sakarkar DM. Stability Indicating RP-HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation. Asian Journal of Research in Chemistry. 2008;1:15-18.
10. Galande VR, Baheti KG, Indraksha S, Dehghan MH. Estimation of amlodipine besylate, valsartan and hydrochlorothiazide in bulk mixture and tablet by UV spectrophotometry. Indian journal of pharmaceutical sciences. 2011;74:18.
DOI: https://doi.org/10.4103/0250-474X.102538
11. Liu F, Zhang J, Xu Y, Gao S, Guo Q. Simultaneous determination of hydrochlorothiazide and valsartan in human plasma by liquid chromatography/tandem mass spectrometry. Analytical Letters. 2008;41:1348-65.
DOI: https://doi.org/10.1080/00032710802119186
12. Thanusha G, Jose C, Babu G, Basavaraj KPC, Panditi VR, Sharadha C. Validated RP-HPLC method for the quantitative estimation of valsartan in bulk and pharmaceutical dosage forms. International Journal of ChemTech Research. 2010;2:1194-98.
13. Kumar PVS, Sahu M, Prasad KD, Shekhar MC. Development and validation of analytical method for the estimation of valsartan in pure and tablet dosage form by RP-HPLC method. Int. J. Res. Pharm. Chem. 2011;1:945-49.
14. Vinzuda DU, Sailor GU, Sheth NR. RP-HPLC method for determination of valsartan in tablet dosage form. International Journal of ChemTech Research. 2010;2:1461-67.
15. Kendre MD, Banerjee SK. Precise and accurate RP-HPLC method development for quantification of valsartan in tablet dosage form. International Journal of Pharmaceutical Sciences and Drug Research. 2012;4:137-39.
16. Gonzalez L, Lopez JA, Alonso RM, Jimenez RM. Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection. Journal of Chromatography A. 2002;949:49-60.
DOI: https://doi.org/10.1016/S0021-9673(01)01496-0
17. Lalitha G, Salomi P, Ravindra RK. Development of an analytical method and its validation for the analysis of atenolol in tablet dosage form by UV-Spectrophotometry. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5: 197–99.
18. Attia KA-SM, Nassar MW, Abolmagd E. Simultaneous spectrophotometric determination of amlodipine and atenolol in pharmaceutical preparations using chemometric techniques. ACAIJ. 2016;16:205-10.
19. Lalitha KV, Kiranjyothi R, Padma B. UV Spectrophotometric method development and validation for the determination of Atenolol and Losartan Potassium by Q-analysis. International Bulletin of Drug Research. 2013;3:54-62.
20. Agarwal R, Gfadnis A. Kinetic spectrophotometric determination of atenolol in perchloric acid medium. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4:350-52.
21. Basavaiah K, Chandrashekar U, NagegowdaP. Titrimetric, spectrophotometric and kinetic methods for the assay of atenolol using bromate-bromide and methyl orange. Journal of the Serbian Chemical Society. 2006;71:553-63.
DOI: https://doi.org/10.2298/JSC0605553B
22. Pai NR, Patil SS. Development and validation of liquid chromatographic method for atenolol and its related substance. Der Pharm. Sin. 2013;4:76-84.
23.Naikini P, Akula A, Ajitha A, Rao VUM. RP-HPLC method development and validation for the simultaneous estimation of amlodipine and atenolol in bulk and tablet dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6:390-94.
24. Chaudhari V, Hussian S, Ubale M. A newer validated and stability indicating HPLC method for the estimation of Atenolol and Hydrochlorothiazide in bulk drug and dosage form. International Journal of Chemical Studies. 2013;1:93-101.
25. Belal F, Sharaf M, Aly F, Hefnawy M, Awady M. Stability-indicating HPLC Method for the Determination of Atenolol in Pharmaceutical Preparations. J Chromat Separation Techniq. 2013;4:1-7.
26. Yilmaz B, Arslan S, Determination of atenolol in human urine by gas chromatography-mass spectrometry method. Journal of chromatographic science. 2011;49:365-69.
DOI: https://doi.org/10.1093/chromsci/49.5.365
27. Tengli AR, Gurupadayya BM. Method development and validation of tablet dosage form containing losartan, atenolol and hydrochlorthiazide using internal standard by RP-HPLC. J Chromat Separation Techniq. 2013;4:1-5.
28. Gradman AH, Basile JN, Carter BL, Bakris GL. American Society of Hypertension Writing, G., Combination therapy in hypertension. Journal of the American Society of Hypertension. 2010;4:90-98.
DOI: https://doi.org/10.1016/j.jash.2010.03.001

29. Hanson R, Gray V. Handbook on Dissolution Testing. 3rd ed. Publisher: Dissolution Technologiesgies, USA, 2004. 199 p.

30. Guidance for industry: Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System / U.S. Department of Health and Human Services, Food and Drug Administration (HHS-FDA). Center for Drug Evaluation and Research (CDER). 2017. URL: https://www.fda.gov/media/70963/download

DOI: http://academy.gmp-compliance.org/guidemgr/files/UCM070246.PDF.

31. Popy FA, Dewan I, Parvin MN, Islam SMA. Evaluation of in vitro equivalence for tablets containing the poorly water-soluble compound atorvastatin. Dissol Technol. 2012;19(4):30-33.

DOI: https://doi.org/10.14227/DT190412P30

32. Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001;13:123–33.

DOI: https://doi.org/10.1016/S0928-0987(01)00095-1

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QUALITY OF LIFE AND WELL-BEING OF POPULATION AT THE END OF THIRD PHASE OF LOCKDOWN IN INDIA AGAINST THE COVID-19 PANDEMIC

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Background. *On March 24, 2020, a nationwide Lockdown for 21 days was ordered by the Government of India which was then extended till May 31, 2020. Researchers have predicted lockdown is a necessary step to prevent COVID-19 spread. However, others have also stated that it could cause serious damage to the economic, mental, social, and physical well-being of the people.*

Objective. *The aim of the study is to evaluate the impact of lockdown on the quality of life and well-being of the Indians.*

Methods. *It is a cross sectional prospective web-based questionnaire study. A link (https://forms.gle/pX25VuahP5NxT88QA) was created. Total 426 responses were received via that link and the data was included in the statistical analysis.*

Results. *Our study revealed that during the lockdown 61.5% of the respondents were performing physical activities lesser than before. More than half responded they had a reduced financial satisfaction. Most answers on emotional well-being and social-family wellbeing were also positive, but some responses showed disturbing too, like 22% felt anxious and nervous over half of the days. It was found in the study that physical, financial, emotional, mental, social and family wellbeing were disturbed during the lockdown and quality of life was also hampered.*

Conclusion. *Though, may be Nationwide Lockdown was the most required action at that point of time to prevent virus spread, but our study revealed that uncertainty regarding its cure and management guidelines like lockdown and social distancing has badly affected quality of life and wellbeing of the population.*

KEYWORDS: **pandemic; lockdown; COVID-19; anxiety; well-being.**

Introduction

In December 2019, several cases of a disease having similar symptoms of pneumonia were reported in Wuhan city of China [1]. World Health Organisation (WHO) defined this disease as COVID-19. Genetically this virus is similar to severe acute respiratory syndrome corona virus (SARS-CoV). SARS CoV-2 strain is the causative agent for COVID-19. Patients of COVID-19 commonly present with symptoms of fatigue, cough, fever, myalgia, and diarrhoea. After China, this virus started spreading to the rest of the world. Its mode of transmission is inhalation of infectious aerosol. Reports revealed that COVID-19 transmission is possible through infected human contact. Due to interhuman transmission, soon it has become global health emergency worldwide. Because of its spread in 144 countries across five continents, the World Health Organisation declared COVID-19 as pandemic disease on March 12, 2020 [2].

By the end of November 2020 this pandemic has infected 70 million people worldwide and the number is increasing day by day. Like the rest of the world COVD-19 has been reported in India too. In India 9.3 million of population has been diagnosed with COVID-19 positive by November 2020. Meanwhile no drug therapy has been established for its prevention, control and cure till now. So, to deal with this Pandemic strict quarantine and lockdown are considered to be a highly effective and important preventive measure by almost the whole world. Following the footsteps, India also took the help of lockdown due to increasing number of cases of COVID-19. Nation underwent 4 phases of lockdown for nearly 70 days.

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The 1st phase of lockdown started on March 24, 2020, for 21 days. Then, an increasing number of cases and severity of the disease forced Government to further extend it into phase 2 for 19 days, and phase 3 for 14 days. From May 18 phase 4 was announced which was planned to end on May 31, 2020. Strict guidelines were formulated to prevent its spread. Only essential services like medical and groceries services were allowed to keep open. Apart from the mentioned services everything else was closed. Based on the number of the cases in particular region, country was divided into 3 zones during the lockdown: green, orange, red.

Green zone covered the areas with zero confirmed cases till date or no confirmed cases in the past 21 days. Orange zone involved the areas, which reported a limited number of cases in the past and no surge of positive cases in recent times. Red zone is for the areas or hotspots classified as those with the highest caseload.

Although, the Lockdown was considered necessary to prevent COVID-19 spread. During the period of lockdown Indian residents are advised to stay at home. It hampered resident's life style very much. Some researchers stated this caused serious damage to emotional [3] economic, mental, psychological [4], social and physical well-being of the population. Due to a prolonged lockdown and business closure, people experienced negative emotions, stress, aggressiveness and anxiety symptoms. So, this study was aimed to evaluate the impact of lockdown on the quality of life and wellbeing of Indians.

Methods

A cross-sectional web-based online survey was conducted for a period of two weeks starting just after the completion of third phase of lockdown in India, from May 25 to June 1, 2020. A survey link (https://forms.gle/pX25VuahP5NxT88QA) was created through a web-based Google application of 'Google Form'. All Indian citizens above the age of 18 years old, who gave an informed consent for participation in the study, were included while NRI and foreign citizens were excluded. Participants were recruited by sending the survey link through various social network channels such as WhatsApp, LinkedIn, Instagram, and Facebook. The final sample was obtained using the snowball technique wherein each participant was requested to further circulate the survey link among their respective family members, friends, and colleagues. The obtained data were analysed.

Study tools counted in a pre-validated 47-item online questionnaire, which was validated for relevance, clarity, simplicity, and ambiguity by using 4- and 5-point content validity index. An informed consent document comprising the participant information sheet and informed consent form in Hindi and English was suggested in the beginning of the questionnaire and only those participants, who gave their consents, were allowed further access to the questionnaire. The questions were in both languages in the questionnaire. The variables and instruments included in the questionnaire comprise the following:

1. **Section 1** with 13 questions on demographics of the participants including age, gender, marital status, educational and professional details, area of residence and its COVID zone, and present state of health.

2. **Section 2** with 34 questions for the assessment of physical (02 questions), psychological (09 questions), financial (07 questions), emotional (06 questions), and social and family well-being (05 questions) of the participants and their quality of life (05 questions).

Questions related to physical wellness were generated ad hoc. For psychological well-being among the participants two tools were used, i.e. the Patient Health Questionnaire-2 (PHQ-2) [5] to screen for depression, and the Generalized Anxiety Disorder Scale (GAD-7) [6] to screen for anxiety. Both the tools consisted of 2 Likert type questions, each with 4 response options ranging from 0-3. The PHQ-2 score ranged from 0-6 with 3 as the optimal cut point while the GAD-7 score ranged from 0-21 with a score of 10 or higher indicates significant anxiety.

Financial well-being was evaluated using a modified COST-FACIT (Version 2) consisting of 7 questions, 6 which were Likert type questions with 5 response alternatives ranging from 0-4. FACIT-Sp (Version 4) was used to assess the physical, social/family and emotional well-being of the participants. For emotional well-being 6 Likert type questions and for social and family well-being 5 Likert type questions were asked, each with 5 response options ranging from 0-4. The WHO (Five) Well-Being Index (1998 version) consisting of 5 Likert type questions having 6 possible options was used to evaluate the quality of life of the study participants during the lockdown. The raw score ranged from 0 to 25; 0 representing the worst possible and 25 representing the best possible quality of life.

Results

A total of 426 responses were received via the study link (https://forms.gle/pX25VuahP5NxT88QA) of 'Google Form'. 421 participants gave their consent for participation and were included in the survey. Their demographic details are depicted in Table 1.

4.8% of the participants responded that they were suffering from chronic health problems, the details are depicted in Fig. 1.

Physical well-being: 23.5% participants responded that during the lockdown, they were able to perform their routine physical activities as they used to do before the starting of lockdown, while 15% responded that they were not able to do so at all, and 61.5% could perform their routine physical activities lesser than before. Health related problems due to changes in daily routine, like drowsiness, weight gain, etc. were experienced by 28.8% participants.

Financial wellbeing (COST FACIT (Version 2)): Regarding satisfaction with their current financial situation consequent to lockdown, majority of the participants (57%) responded

Table 1. Demographic details of the study participants

Variables		No. of responses (percentage)
Gender	Males Females	267 (63%) 154 (36.3%)
Age	18-45 years old 45-60 years old Above 60 years old	328 (97.32%) 8 (2.3%) 1 (0.38%)
Marital status	Unmarried Married Divorced/widowed	261 (61.5%) 158 (37.4%) 04 (1.1%)
Education	Graduate Post-graduate High school Intermediate	232 (54.8%) 154 (36.4%) 5.2% (22) 3.6% (15)
Occupation	Student Service Business Housewife	190 (45.8%) 122 (29.3%) 53 (12.7%) 33 (7.9%)
Type of service	Not applicable Private Government	188 (46.2%) 126 (31%) 93 (22.9%)
Residence	Urban Rural	295 (70.1%) 126 (29.9%)
COVID Zone of the area of residence	Red zone Orange Zone Green Zone Don't know	186 (44.1%) 132 (31.1%) 97 (23.1%) 08 (1.7%)
During lockdown, living with	Family Initially stuck away then able to live with family Away from family	296 (69.7%) 68 (15.8%) 59 (14.4%)
Whether profession is related to COVID frontline fighting	Yes No	79 (19.2%) 343 (80.8%)
Whether suffering from any chronic health problem	Yes No	19 (4.8%) 402 (95.2%)
Preferred to stay home during lockdown because of	Fear of strict government action Fear of getting infected Pressure from family	30 (7.3 %) 342 (83%) 40 (9.7%)
State of health at present	Excellent Very good Good Fair Poor	76 (18.4%) 145 (34.2%) 140 (33%) 51 (11.8%) 12 (2.3%)

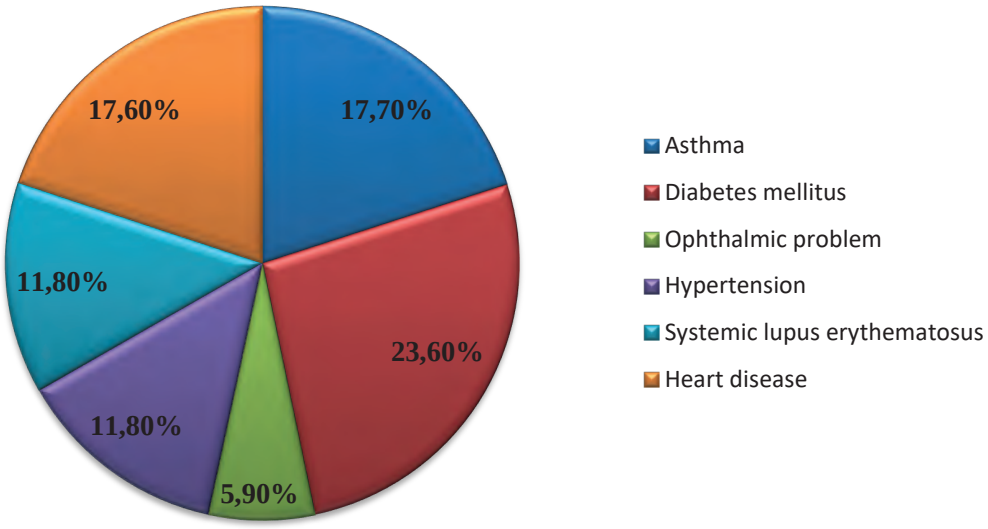

Fig. 1. Distribution of various chronic health problems among the study participants.

that they have a reduced satisfaction, 29.4% were satisfied, and 13.6% felt financially stressed.

Fig. 2 illustrates the responses of the participants to the COST FACIT Likert questions on financial well-being.

Fig. 3 illustrates the responses of the participants to the FACIT-Sp (version 4) Likert questions on emotional Wellbeing.

Fig. 4 illustrates the responses of the participants to the FACIT-Sp (version 4) Likert Social and family well-being.

***Psychological well-being*:** Responses regarding mental well-being are shown in Table 2.

Table 3 depicts the scores for PHQ-2, GAD-7, and WHO well-being index.

Fig. 5 illustrates the responses of the participants on the quality of life (WHO Well-Being Index (1998 version)).

Discussion

The study population consisted of 426 participants: 421 participants were fulfilling inclusion and exclusion criteria. The present

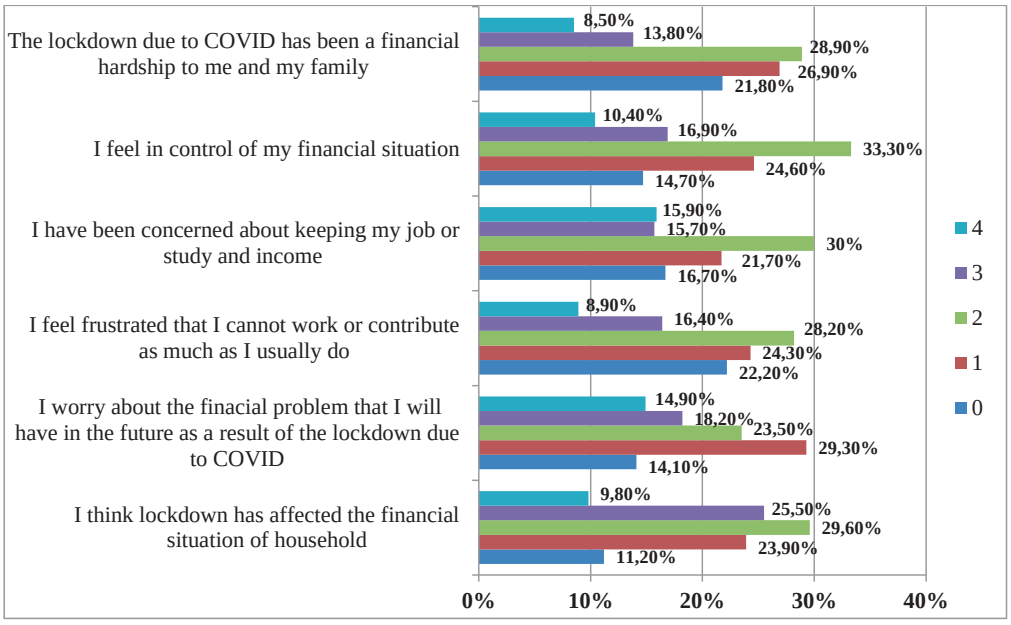

Fig. 2. Participants responses (0 – not at all, 1 – a little bit, 2 – somewhat, 3 – quite a bit, 4 – very much) to statements on financial well-being in COST FACIT (Version 2)

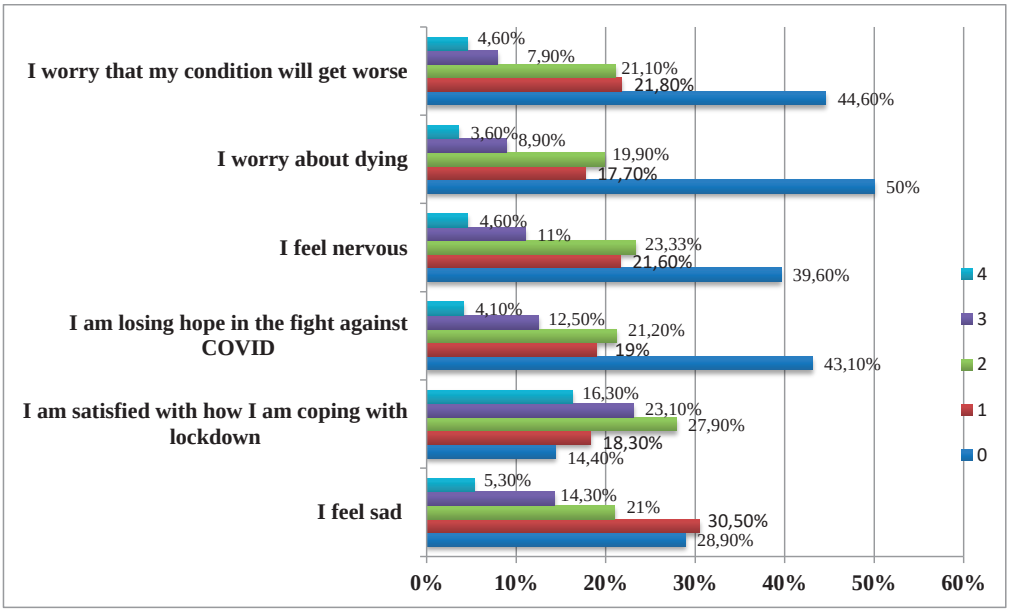

Fig. 3. Participants responses (0 – not at all, 1 – a little bit, 2 – somewhat, 3 – quite a bit, 4 – very much) to statements on emotional well-being in FACIT-Sp (Version 4).

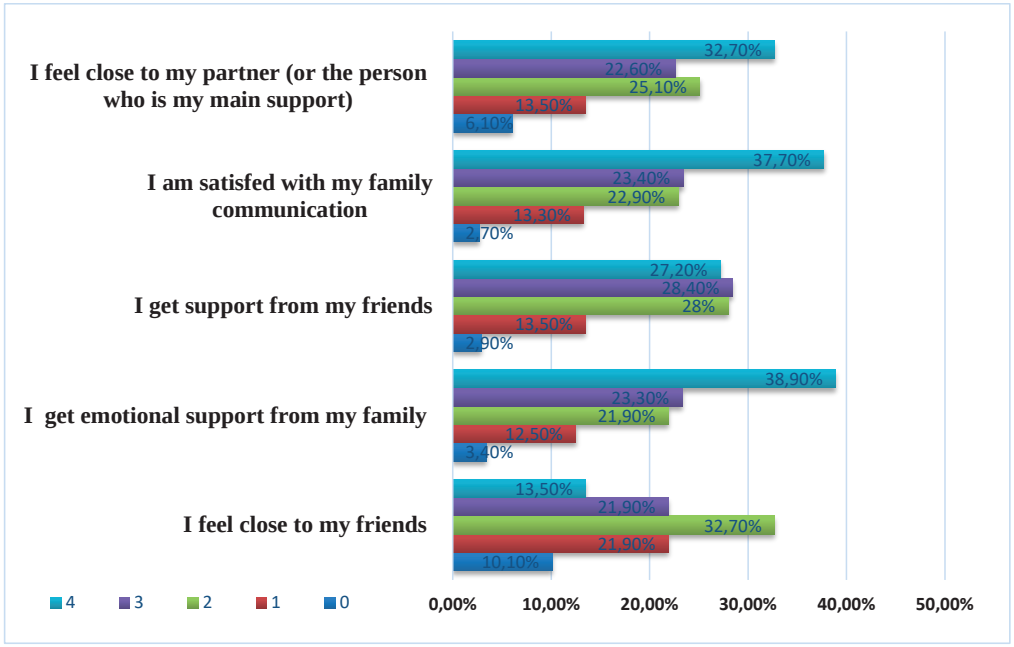

Fig. 4. Participants responses (0 – not at all, 1 – a little bit, 2 – somewhat, 3 – quite a bit, 4 – very much) to statements on social and family well-being in FACIT-Sp (Version 4).

study emphasizes to all types of well-being (physical, psychological, financial, emotional, social, family and quality of life) of Indian population during the lockdown due to spread of COVID-19. Although, the lockdown was thought to be the most effective way to prevent the spread of COVID-19, it has also negatively affected the quality of life and well-being of population.

People faced a lot of physical health related problems during this period. 61.5% study population responded that they were not able to perform their physical activities as before, 28.8 % of the participants felt physical changes in their body. Majority of the participants (39.1%) felt lesser interest in doing things for several days. Similarly, 33.7% felt down depressed for hopeless for several days during

Table 2. Generalized Anxiety Disorder Scale (GAD-7)

	Not at all (0)	Several days (1)	Over half the days (2)	Nearly every day (3)
Feeling nervous, anxious or on edge	37.8 %	34%	22%	6.2%
Not being able to stop or control worrying	35.2%	33.5%	22%	9.3%
Worrying too much about different things	35.2%	32.3%	23%	9.6%
Trouble relaxing	39.1%	31.2%	22.8%	7%
Being so restless that it's hard to sit still	46.4%	26.8%	21.5%	5.3%
Becoming easily annoyed or irritable	37.2%	30.9%	23.7%	8.2%
Feeling afraid as if something awful might happen	38.8%	33.1%	20.1%	7.9%

Table 3. Population distribution of PHQ-2, GAD-7, and WHO well-being index score

Name of Score	Score Range	No. of responses (percentage)
PHQ-2	3–6 0–2	90 (21.12%) 336 (78.88%)
GAD-7	10 or higher Below 10	135 (31.66%) 291 (68.34%)
WHO Well-Being Index	13 or higher Below 13	275 (64.56%) 151 (35.44%)

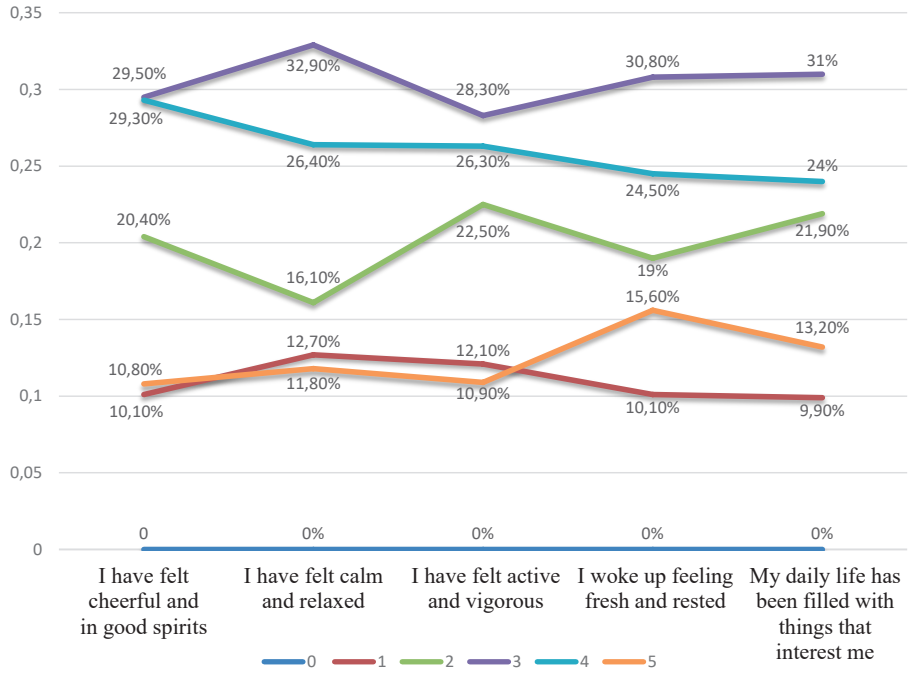

Fig. 5. Participants responses (0 – at no time, 1 – some of the time, 2 – less than half of the time, 3 – more than half of the time, 4 – most of the time, 5 – all the time) to statements on Quality of life (WHO Well-Being Index (1998 version)).

this period. This Pandemic crisis did not only affected the physical well-being but also people were more worried about the financial situation and its impact on their financial status in future. Related to financial well-being, 57% of the population responded that they had a reduced satisfaction with their financial situation and 13.6% felt financially stressed because of this. Out of total, 15.9 % were worried about keeping their job or study which was not only harmful for their financial well-being but also more likely to affect their mental well-being. Majority (28.9%) agreed on the statement that it was somewhat of financial hardship to them and their families.

In our study the most positive responses were observed on emotional well-being. Most answers on emotional well-being were having zero score which indicates population had great control on emotional situations. In similar way, the sections of social and family wellbeing were also answered very positively. The participants (38.9%) were very much happy with the support by their families and 37.7% felt satisfied with their communication with families. 32.7% felt very much close to their partners.

Emotional social and family wellbeing were responded positively by the majority of the participants, but the responses by some of the participants to several questions for disturbing too: 22% felt anxious and nervous over half of the days. They were not able to stop worrying. Nearly same percentage of the population felt too much worrying and they were feeling so restless that it was hard for them to sit or relax. Almost the same percentage of the population (23.7%) became easily annoyed or irritable. They (20.1%) felt afraid as a something awful might happen. However, these types of responses were given by 22% of population but still it is disturbing because it may have led to mental stress and mental health related issues to them. Out of total population, 29 to 32% felt cheerful, calm, relaxed, active, vigorous and fresh for more than half of the time.

Nevertheless, the results are not encouraging in terms of overall wellbeing of the population. Our findings indicate the need of serious attention on the quality of life and wellbeing of the population due to changes in lifestyle during the COVID-19 lockdown. It would be a huge challenge for not only the individuals to regain their physical, financial, emotional, mental, social and family wellbeing again but also for the government of India to re-establish the financial condition of the country by coping this pandemic crisis.

Limitations. Although, our study tried to involve population of all social economic and educational status, due to web-based study it wasn't feasible for the individuals of all socioeconomic status to take part in this study. Apart from this, shorter time span was also its one of the limitations. So, studies including larger sample size can be conducted.

Conclusion

Our findings suggest that lockdown has affected various aspects of life of each and every individual of the country. People are dealing this Pandemic with all their efforts but anxiety regarding future is making them weaker. Uncertainty of prevention and treatment of SARS-CoV2 is the major drawback to keep up the good spirit. The Lockdown has the transient benefit for prevention of spread but not a permanent solution to this problem.

Conflict of Interests

Authors declare no conflict of interest regarding this study.

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Author's Contributions

Heena Rathi, Priyanka Rathi – conceptualization, methodology, formal analysis, writing – original draft, writing – reviewing and editing; *Heena Rathi, Mohit Biyani* – data curation, writing – reviewing and editing; *Manisha Malik, Mohit Biyani* – investigation; *Manisha Malik* – formal analysis.

ЯКІСТЬ ЖИТТЯ НАСЕЛЕННЯ ІНДІЇ В КІНЦІ ТРЕТЬОГО ЛОКДАУНУ, СПРИЧИНЕНОГО ПАНДЕМІЄЮ COVID-19

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Вступ. *24 березня 2020 року уряд Індії впровадив загальнодержавний локдаун на 21 день, який потім було продовжено до 31 травня 2020 року. Дослідники передбачають, що обмеження пересування є необхідним кроком для запобігання поширенню COVID-19. Однак також відомо, що це може завдати серйозної шкоди економічному, психічному, соціальному та фізичному благополуччю людей.*

Мета. *Завдання цього дослідження – оцінити вплив локдауну на якість життя та добробут населення Індії.*

Методи. *Дослідження проводилося методом проспективного поперечного перерізу шляхом веб-опитування. Було створено посилання (https://forms.gle/pX25VuahP5NxT88QA). Всього було отримано 426 відповідей за посиланням, і ці дані були проаналізовані.*

Результати. *Дослідження показало, що 61,5% респондентів мали менше фізичне навантаження під час локдауну. Ще 57% відповіли, що не задоволені своїм фінансовим становищем. Відповідь більшості мала позитивне забарвлення щодо емоційного благополуччя та соціально-сімейного благополуччя, але відповіді деяких опитаних мали тривожний характер: 22% відчували занепокоєння та нервозність протягом майже всього періоду локдауну. Наше дослідження виявило, що фізичне, фінансове, емоційне, психічне, соціальне та сімейне благополуччя порушуються під час локдауну, також страждає якість життя.*

Висновок. *Незважаючи на те, що загальнодержавний локдаун, можливо і був найбільш необхідною дією для запобігання розповсюдженню вірусу, але наше дослідження показало, що невизначеність відносно лікування інфекції та рекомендацій щодо локдауну та соціального дистанціювання, мала значний вплив на якість життя та добробут населення.*

КЛЮЧОВІ СЛОВА: **пандемія; локдаун; COVID-19; тривога; якість життя.**

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References

1. Maugeri G, Castrogiovanni P, Battaglia G, Pippi R, D'Agata V, Palma A, Di Rosa M, Musumeci G. The impact of physical activity on psychological health during Covid-19 pandemic in Italy. Heliyon. 2020 Jun 1;6(6):e04315.
DOI: https://doi.org/10.1016/j.heliyon.2020.e04315

2. Lesser IA, Nienhuis CP. The impact of COVID-19 on physical activity behavior and well-being of Canadians. International journal of environmental research and public health. 2020 Jan;17(11):3899.
DOI: https://doi.org/10.3390/ijerph17113899

3. Lades LK, Laffan K, Daly M, Delaney L. Daily emotional well-being during the COVID-19 pandemic. British journal of health psychology. 2020 Nov;25(4):902-11.
DOI: https://doi.org/10.1111/bjhp.12450

4. Rodríguez-Rey R, Garrido-Hernansaiz H, Collado S. Psychological impact and associated factors during the initial stage of the coronavirus (COVID-19) pandemic among the general population in Spain. Frontiers in psychology. 2020 Jun 23;11:1540.
DOI: https://doi.org/10.3389/fpsyg.2020.01540

5. Kroenke K, Spitzer RL, Williams JB. The Patient Health Questionnaire-2: validity of a two-item depression screener. Medical care. 2003 Nov 1:1284-92.
DOI: https://doi.org/10.1097/01.MLR.0000093487.78664.3C

6. Spitzer RL, Kroenke K, Williams JB, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. Archives of internal medicine. 2006 May 22;166(10):1092-7.
DOI: https://doi.org/10.1001/archinte.166.10.1092

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HISTOMORPHOMETRIC ESTIMATION OF AGE FROM BONE SAMPLES OF NIGERIANS

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Background. *Age estimation is crucial in creating the biological profile of unknown skeletal remains and recently there is emphasis on the need to develop population specific forensic baseline data for easy identification of these remains.*

Objective. *The aim of this study is to estimate age from the histomorphometric features of the bones of Nigerians.*

Methods. *Fragments of non-pathologic bone samples were collected during orthopaedic procedures. Ground sections were prepared using Modified Frost's manual method of bone preparation to determine the following histologic parameters; haversian canal diameter (HCD), primary and secondary osteons, number of osteon fragments and non-haversian canal as well as haversian canal area (HCA). 29 subjects aged 35 to 85 years old were used for the study. Bone fragments included samples from the femur, tibia, humerus, and the vertebrae. Data obtained were subjected to descriptive statistics, Pearson's correlation, bivariate regression equation, Student t-test and analysis of variance (ANOVA).*

Results. *The mean age for our population was 58.86 years old. ANOVA showed significant variation in the average HCD for the various regions: humerus=8.45±2.48, femur=7.09±4.06, tibia=8.70±2.52 and vertebrae=3.69±0.73. There was a strong inverse relationship between age and primary osteons. The total number of osteon fragments increased with age while total number of primary osteons and average HCD decreased with age. The HCA, though statistically insignificant, also decreased with age.*

Conclusion. *Our findings show that three histomorphometric parameters showed significant correlation with age: osteon fragments (OS-f), primary osteons (OS-p) and HCD. The histomorphometric parameters were therefore relevant in age estimation.*

KEYWORDS: **age estimation; histomorphometric parameters; Nigerians; forensics.**

Introduction

Age estimation from the macrostructure of bones is an aged-long anatomical practice, which has progressed into studying the micro structural features. Hence, it is an important tool in physical anthropology as well as in forensic medicine in the identification of skeletal remains. Once age and sex are estimated, the identity of the skeletal remains is established for about 80% [1, 2, 3].

There are few methods used by forensic scientists whenever the issue of age assessment arises and as such several approaches have been developed. These methods include the use of dental eruptions periods [4, 5, 6], time of epiphyseal fusion of some bones and microscopic or histological features of cortical bones [7, 8, 9], morphology of the pubic symphysis and auricular surface of the Ilium [10, 11, 12] the skull and length of long bones [13, 6, 7], sternal ends of the 3rd, 4th and 5th ribs [14, 15]. These foremost macrostructural approaches have lots of limitations as only some bones like the pelvic bone and some long bones give reliable results [16]. These approaches also require the researcher to employ wide age ranges during age assessment of the subjects that would introduce major difficulties in age estimation of specimen [16, 17].

In order to overcome the limitations posed by the macroscopic methods, in 1965 Kerley developed the quantification of histological features of cortical bone for age estimation [18, 19, 20, 21]. Since bone growth, development and changes occurs with age, it was presumed that the study of the histological features of

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bones could be important in the estimation of age for human populations [22, 23, 21, 24, 25]. According to De Boer and Maat (2003) [26] and Thomas et al. (2000) [27], the histological findings must be combined with gross anatomical and radiological findings to obtain a conclusive diagnosis or to shorten a list of differential diagnosis.

Singh and Gunberg (1970) [22] and Wolf et al. (2017) [28] demonstrated the use of fragmentary remains of bone to determine age from histology. Some studies from different populations have used the quantification of osteon in age estimation [29, 30, 31]. A common finding of these studies is that the number of osteon and diameter of the Haversian canal changes with age [2, 18, 32, 33]. Steyn et al. (2004) [33] also observed that age estimation was easy in children compared to adults due to bone remodeling and degeneration, and became more difficult in older ages. Currently, it has been established that there is need to generate forensic data for age, sex and stature estimation for different populations, as anatomical landmarks are influenced by diet, disease, genes, sex and racial peculiarities [16, 34]. This study therefore seeks to generate baseline data for age estimation from bone histology among Nigerians.

Methods

This is a descriptive and correlational study involving life subjects. Fragments of bone samples were collected during orthopedic procedures with data on the age, sex, tribe and type of pathology or clinical diagnosis of the subjects. Bone samples collection and tissue preparation took a period of about six months. Samples were collected from the Orthopedic departments of The University of Port Harcourt Teaching Hospital (UPTH) and Rivers State University Teaching Hospital (RSUTH), as well as Rehoboth Specialist Hospital Port Harcourt and Twin Towers Specialist Hospital Port Harcourt. Ethical Approval

Ethical approval was sought from the University of Port Harcourt Research Ethics committee and was granted. A consent form was issued to each patient and an informed consent was obtained after thorough guidance and counselling of the patient.

Sample was drawn from orthopedic patients with no prior or background metabolic disease. Bone fragments were collected from amputations and other orthopedic procedures where bone fragment**s** could be harvested. A total number of 29 subjects with samples from 12 females and 17 males aged 35 to 85 years old were used for study. Bone fragments included samples from the femur, tibia, humerus, and the vertebrae. The samples included healthy and strong bone fragments without any periosteal disruption. Diseased bone fragments shattered during grinding were excluded during processing. The sampling technique involved a convenience sampling method. The bone fragments were collected and ground sections were prepared using the Modified Frost's manual method of bone preparation [35, 36, 16]. Bone fragments were collected in properly labeled plain containers during operations and immersed in water for about 1 week to enable soft tissue removal. Some of the soft tissues were manually removed gently to prevent distortion of the periosteal layer of the bones. The samples were subsequently fixed in 10% formalin for one more week. The volume of fixative is 10-20x the size of the bone to allow adequate penetration of fixative.

With the help of a hacksaw, thin cross sections of bone fragments were made. A glass slab coated with Vaseline was prepared. A p220 sand paper was placed on the slab. The application of Vaseline helps prevent moisture and allows close and smooth adherence of the sand paper to the glass plate. This would allow proper and concurrent thinning of all edges of the bones during subsequent grinding. The rectangular glass slab measured 16cm×12cm and the sand paper was cut to a little more than the size of the glass slab. This would prevent water from escaping into the underside of the paper. A drop of water was dropped on the sand paper and the Modified Frost's manual method of bone preparation was adopted. This method was implemented owing to the distortion of micro architecture of tissues with decalcification before routine histology. Gentle grinding of bone sections was done by moving the pulp of the finger on the bone over the sand paper in a cyclical fashion. This would enable thinning uniformity of the bone edges. This would also prevent easy cracking and breaking of sections as they get thinner. Water was added continually during grinding progresses to enable lubrication and reduce of friction. Physical observation of sections for thinness was done by means of a tweezer and a fine brush. With the help of frost's holder, very thin sections were held for grinding until required degree of thinness was attained. During the grinding process, utmost care was taken to avoid scratching away of the

periosteum. Hence bone sections were turned from one surface to another to allow for evenness of thinning. Grinding was done until bone sections appeared opaque, and then transparent and even easily bendable using the fine brush. Ready specimens were placed in a beaker containing distil water and a little drop of detergent in order to properly rinse out dirt and stains from the specimens. Washing was performed by the tweezer and the fine brush. Rinsed specimens were then placed on a filter paper in a Petri dish to allow for drying of the specimen. The filter paper was gently roughened before use. This allowed for ease of pick of the section with the fine brush after drying. Glass slides were then cleaned with absolute alcohol and then placed on a glass slab having a dark background. This was achieved by placing a black sheet of paper or polythene behind the glass slab. A drop of DPX mountant was placed on the glass slides. The dried specimen was placed on a top of the mountant. Another drop of the mountant was immediately dropped on the specimen to allow proper immersion of the specimen. Glass cover slips washed in xylene were placed over the specimen. The mountant was seen to spread evenly from under the cover slip. Air bubbles trapped under the cover slip in some specimens were removed by gentle pressure of the cover slip with the tweezer. Ready slides were allowed to dry and correctly labelled. They were kept to dry in a horizontal position for about 24 hours before loading into glass boxes. Ready slides were mounted under a photomicroscope for viewing and analysis. The Leica ICC 50E photomicroscope was used to view and demonstrate the histological features. Photomicrographs of fields adjudged by two researchers to have more osteon density were taken. This was done after thorough review and examination of the entire field under study.

Results

Table 1 shows the various histomorphometric parameters studied. Their mean distribution as well as the minimum and maximum values for each parameter is also presented. The average age distribution for sample population is shown as well.

Table 2 shows the mean age, average HCD, standard deviation and variance of the Haversian canal diameter of various bones studied. The average HCD for the various regions is as follows: humerus=8.45±2.48, femur=7.09±4.06, tibia=8.70±2.52 and vertebrae=3.69±0.73.

Table 3 demonstrates the use of ANOVA in determining the degree of variation in the HCD of the various bones studied. The F value is greater than the F critical for an alpha level of 0.05. This proves that there is a significant variation between the mean of the various Haversian canal diameter of the bones studied. The P value obtained here (P=0.00) is also less than the alpha level chosen (0.05). This evidences that the variation in the Haversian canal diameter of the various bones is very significant.

Table 4 presents the regression equation derived for age estimation using the various

Table 1. Descriptive Statistics of Age and bone histomorphometric variables

Parameters	Mean	Standard error of mean	Standard deviation	Variation	Minimum variation	Maximum variation
Age	58.86	3.52	16.50	272.12	35.00	85.00
OS-p	2.14	0.58	2.71	7.36	0.00	11.00
OS-s	2.45	0.83	3.89	15.12	0.00	12.00
OS-f	7.00	1.29	6.04	36.48	0.00	22.00
N-hc	2.32	0.47	2.19	4.80	0.00	7.00
Area HC	54.13	4.70	68.71	3446.74	3.53	534.35
HCD	7.53	0.28	3.51	12.31	2.19	26. 08

Notes: OS-p=primary osteon, OS-s=secondary osteon, OS-f=osteon fragment, N-hc=Non haversian canal, HC=Haversian canal, HCD=Haversian canal diameter.

Table 2. The Average Haversian canal diameter for the different bones

Groups	Mean age (years)	Counts	Sum	Average (microns)	Standard deviation
Humerus	67	31	262.06	8.453484	2.48
Femur	60.71	41	356.51	7.090278	4.06
Tibia	55.33	72	510.50	8.695366	2.52
Vertebrae	51	11	40.60	3.690909	0.73

Table 3. Test of Variation in HCD using ANOVA

Source of Variation	SS	df	MS	F value	P–value (P<0.05)	F critical
Between groups	258.1242	3	86.0414	7.905552	0.00**	2.664504
Within groups	1643.434	151	10.88367			
Total	1901.558	154				

*Notes: HCD – Haversian canal diameter, ** – very significant.*

Table 4. Correlation, coefficient of determination and regression equation for age versus bone histomorphometric variables

Parameters	Correlation coefficient®	Coefficient of determination	t value	P value	Regression equation
OS-p	0.37	0.14	1.78	0.09	y=-2.29x+63.75
OS-s	0.17	0.03	0.77	0.45	y=0.71x+60.59
OS-f	0.30	0.09	1.41	0.17	y=0.812x+53.18
N-hc	0.10	0.01	0.45	0.66	y=0.8926x+50.79
Area HC	0.24	0.056	1.10	0.28	Y=-0.0568x+58.896
HCD	0.26	0.069	1.21	0.24	Y=-1.0554x+63.768

Notes: OS-p=primary osteon, OS-s=secondary osteon, OS-f=osteon fragment, N-hc=Non Haversian canal, HC=Haversian canal, HCD=Haversian canal diameter, Y=Predicted Age, x=Parameter under consideration.

histomorphometric parameters. It also shows the correlation coefficient and coefficient of determination as well as the t and p values of the variables.

Discussion

Our study considered the correlation between age and histological parameters such as primary osteons (OS-p), secondary osteons (OS-s), and osteon fragments (OS-f), non-haversian canals (n-HC), Haversian canal area (HCA) and Haversian canal diameter (HCD). The mean age for our total population was 58.86 years old (Table 1). The average HCD also appeared to vary with the region of bone chosen as we obtained 8.45 microns for humerus, 8.70 microns for tibia, 7.09 microns for femur and 3.69 microns for the vertebra (Table 2).

It is also possible that bone density contributed to the size of Haversian canal as demonstrated with the vertebrae. Although the mean ages for tibia and vertebrae are similar, the variation in HCD is large, and more so when compared with the femur and humerus. ANOVA test also shows that there is a significant variation between the mean of the various Haversian canal diameter of the bones studied (F value > F crit. at alpha level 0.05) (Table 3). The P-value (P=0) at alpha level 0.05 is also less than the alpha level (Table 3). This proves that variation in the HCD of the various bones is significant (P<0.05). These differences seen with different regions could be due to varying bone activity and bone density. It is supposed therefore that age and aging could not be the major reason for these regional differences. The vertebra is not exposed to too much stress and activity as compared to the long bones, especially the tibia, which is a major weight bearing bone. Whether these findings could differ with occupation and especially in gymnasts is yet to be ascertained. According to Keough (2007) [16], the average Haversian canal diameter (HCD) ranges at 30-70 microns. This is quite at variance with our values as our study obtained a range of approximately 2.2–26.1 microns. The reasons also could be the type of sample used. Keough used samples from femoral mid-shaft from a predominantly black South African population and being within a similar mean age to ours. Also, Singh and Gunberg (1970) [22] in a study on bone fragments of male American population of similar age group discovered that HCD varies with region of bone chosen for the study and obtained average HCD of 63.44 microns for the mandible fragments, 43.24 microns for the femur and 45.54 microns for the tibia. Both previous studies used skeletal collections from cadavers of a white and some black population whereas our study used skeletal remains of live humans from Nigerian population. Whether the wide variation is related to the tribe or the type of sample used is yet to be determined. However, it is obvious

that Nigerians may have far lower average HCD than other populations studied. Our study discovered that HCD decreases as age increases (Fig. 1).

This agrees with Singh and Gunberg, (1970) [22] who studied American population and reported that HCD decreases as age increases. They also established strong correlation with age. They proved that between age of 40-45 years old the HCD could range between 85-92 microns, and could fall to a range of 51-58 microns at age of 70-80 years old. Though we obtained smaller HCD for the Nigerian population, our sample was collected largely from an older age group of between 55-85 years old, hence the reason to have obtained small HCD for age. Whether ethnicity, socioeconomic status, diet or undiagnosed disease may have an impact, this outcome is yet to be ascertained as it is not within the scope of this study. Landeros and Frost (1964) [37] also proved that closure of the Haversian canal continues as age increases, hence consenting to a reduced diameter with age. However, Keough (2007) [16] reported a slight positive correlation (r= 0.1377) with age and supposed males showed highest correlation with age (r=0.9964). He also noted that this parameter could not be a strong estimator of age. Likewise Sobol et al, (2014) [9] established that HCD increased with age and assumed it was one of the best predictors of age. But Barer and Josey, (1967) [38] reported little or no age-related changes in the size of the Haversian canal. As age increases, the HC area decreases (Fig. 2).

The coefficient of determination (r^2= 0.056) was quite small (Table 4), showing that only 5% variation in age of the subjects can be explained by the total Haversian canal area of the samples. Hence though there is a correlation with age, the coefficient of determination shows that this parameter may be weak in age estimation. The HCD thus has a stronger correlation with age compared to the Haversian canal area. The HCD is therefore a better estimator of age compared to the Haversian canal area.

Fig. 3 shows a strong inverse relationship between age and primary osteons (OS-p). The younger age groups appear to have more number of primary osteons.

This is in consent with Enlow (1963) [39] and Keough, (2007) [16] who have established that primary osteons is more in the younger age group. Non Haversian canal shows no correlation with age (r= 0.10) (Fig. 4).

This finding also contradicts the reports of many researchers who have proved that N-hc decreases with age [32, 30, 16]. For instance, Ericksen (1991) [32] established that N-hc decreased with age for both sexes. Also, Keough (2007) [16] noted that after 55 years of age, the presence of non Haversian canals ceased almost completely. It is also important to note that Tersigni's (2005) report agrees with our data where he wrote that N-hc showed no significant variation with age [40].

Both primary osteons and non-Haversian canals make up the total number of unmodeled bone. Bone remodeling occurs in response to stressors and graded amount of activity, and

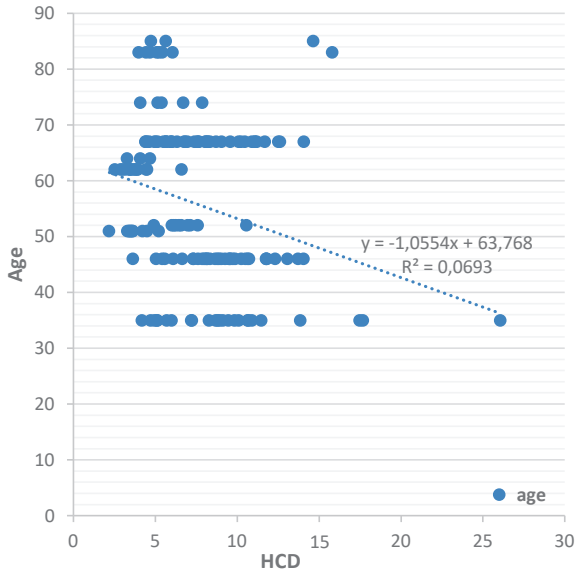

Fig. 1. Scatter plot of Age versus HCD.

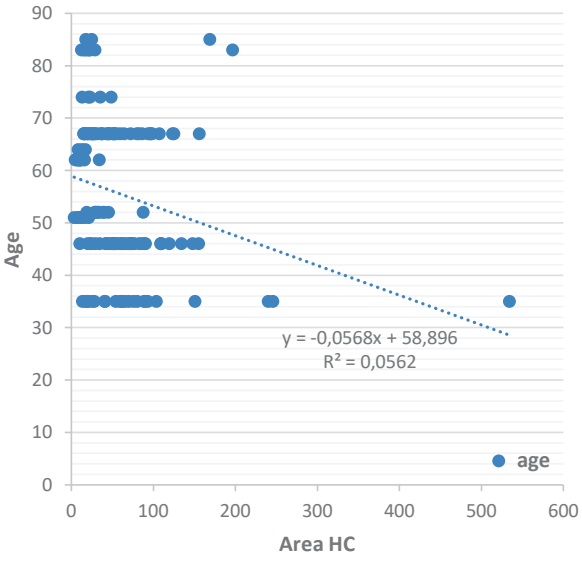

Fig. 2. Scatter plot of Age versus Area-HC.

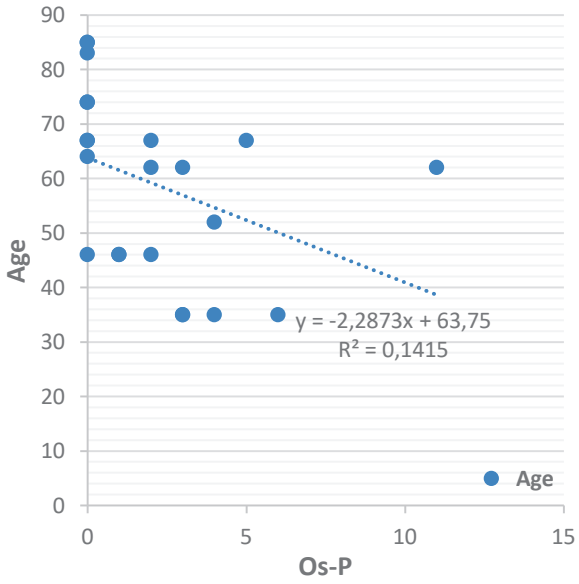

Fig. 3. Scatter plot of Age versus Os-p.

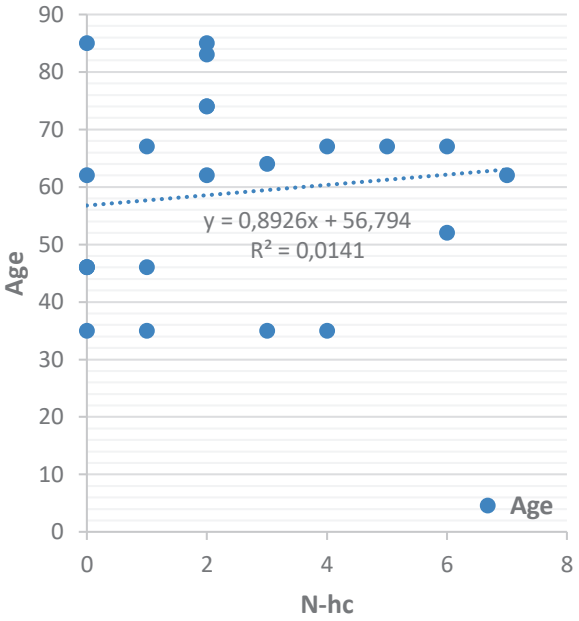

Fig. 4. Scatter plot of Age versus N-hc.

this therefore increases with age. Therefore, finding more primary osteons in the younger age group is justifiable as proved in our research findings. Ingraham (2004) [30] confirmed our findings when he reported that percentage of unremodeled bone is higher in the younger age with 53.4% for 18 years of age and 2.2% for 69 years of age.

Fig. 6 reveals a photomicrograph taking under a magnification of ×100 for two males at ages of 67 and 35 years old. The features seen were predominantly secondary osteons for the older age and unremodeled bone for the younger individual. These findings were expected as bone remodeling increases with age. These findings however are not consistent with all ages in our study; hence the reasons could be for variable underlying factors like sex, tribe, genetics, undiagnosed metabolic disease, exposure to various levels of stress, as well as variation in graded level of activity for different individuals. Osteon fragment in our study has a weak positive correlation with age (r=0.30) and statistically insignificant (p>0.05) (Table 4) (Fig. 5).

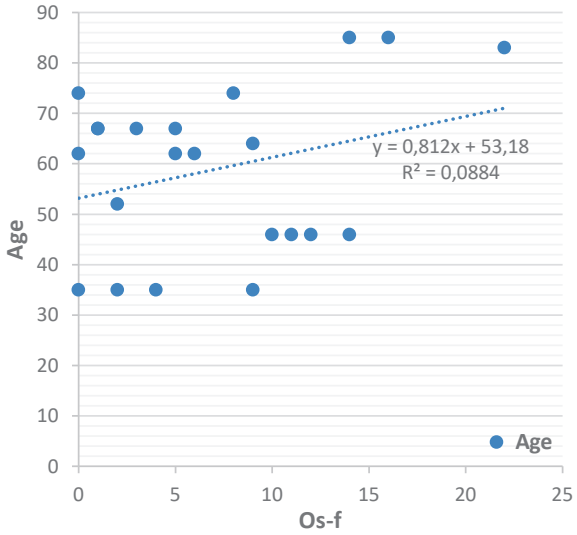

Fig. 5. Scatter plot of Age versus Os-f.

Keough (2007) [16] documented that osteon fragments showed significant correlation with age (r=0.55). Kerley's (1965) [18] novel research on bone histology proved our findings and stated that osteon fragments were best predictors of age using the fibula. This has been proved by our research and by several authors using various other segments of bones. Keough (2007) [16] also established that four of the histomorphometric parameters studied showed significant correlation with age: total osteon count (r=0.53), % unremodeled bone (r=0.53), total N-hc (r=0.55) and % osteon fragment (r=0.55). In his study, total osteon count and percentage osteon fragments increased with age while percentage of unremodeled bone and non Haversian canal decreased with age. Our findings proved that three histomorphometric parameters showed positive correlation with age: osteon fragments (OS-f), primary osteons (OS-p) and the Haversian canal diameter (HCD).

In our study, the total number of osteon fragments increased with age while total number of primary osteons and average Haversian canal diameter decreased with age. The area of Haversian canal, though statistically insignificant, also decreased with age. The number of non-Haversian canal also increased with age but was statistically not significant as well. We can therefore infer that the Haversian canal area, the non-Haversian canals and the secondary osteons show no significant variation with age. Purves et al. (2011) [41], stated in their study that osteon number was very reliable in age estimation but influenced by nutrition, disease, population and sex. Whether these factors had a great impact on our findings is yet to be ascertained as it is not within the scope of this study. Our samples were obtained from clinically healthy subjects. However about 69% of Nigerians live far below the poverty level according to the 2019 global multidimensional poverty index report by UNDP/OPHI [42], it is not without doubt that nutrition could be a factor. Steyn (2004) [33] in her research on adult age estimation reported that age estimation was difficult in adults and more difficult in older ages. Hence, she advised use of multifactorial approach. This would therefore combine both histological and macroscopic findings, especially where both can be readily available. In the absence of whole skeletal collections, the use of fragmentary remains becomes the main stay. However, in order to make this more robust and accurate, any possible finding on culture, environment and data on DNA can make a whole lot of difference.

Study limitations

The availability and access to bone samples is one foremost limitation to this study. Skeletal remains and bone samples are handled with much pessimism in our culture for fear of ritualists; hence processes for obtaining samples were quite laborious and challenging. Another major limitation to our study is the inability to use cadavers for the research. Our use of cadavers may have allowed access to lots of samples but nonetheless most of our cadavers are not profiled unlike what is obtainable in other countries.

Conclusions

The histomorphometric parameters are therefore relevant in age estimation and sex identification. Most forensic case identification without choice needs skeletal remains in order to investigate their victim. Forensic investigation of Nigerians would therefore need the analysis of the primary osteons, the osteon fragments and the Haversian canal diameter as proved in our research. Thus, since histomorphometric variations with population are influenced by environment, diet and genetics, age and aging of an individual is therefore not a major factor in influencing the histological changes seen, especially for individuals who are within a similar age bracket. Hence any forensic case investigation should, if possible and if the data are available, consider the multifactorial approach.

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Conflict of Interest

The authors declare no conflict of interest.

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Author's Contributions

Loveday Oghenemavwe, Clinton David Orupabo – conceptualization, methodology, formal analysis, writing – original draft, writing – reviewing and editing; *Loveday Oghenemavwe, Clinton David Orupabo, Tamunokuro Diamond* – data curation, investigation.

ОЦІНКА ВІКУ ЗА ГІСТОМОРФОМЕТРИЧНИМИ ОСОБЛИВОСТЯМИ КІСТОК НІГЕРІЙЦІВ

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Вступ. *Оцінка віку має вирішальне значення для створення біологічного профілю невідомих скелетних останків, і останнім часом робиться наголос на необхідності розробки специфічних для певної когорти населення судово-медичних даних для легкої ідентифікації цих останків.*

Мета. *Метою цього дослідження була оцінка віку за гістоморфометричними особливостями кісток нігерійців.*

Методи. *Фрагменти непатологічних зразків кісток збиралися під час ортопедичних процедур. Зрізи були підготовлені за допомогою модифікованого ручного методу підготовки кісток для визначення наступних гістологічних параметрів: діаметр каналу Гаверса (HCD), первинний та вторинний остеони, кількість фрагментів остеону та негаверсових каналів, а також площа каналу Гаверса (HCA). Для дослідження було використано 29 досліджуваних у віці від 35 до 85 років. Осколки кісток включали зразки стегнової, гомілкової, плечової кісток та хребців. Отримані дані піддавали описовій статистиці, кореляції Пірсона, двовимірному рівнянню регресії, t-критерію Стьюдента та дисперсійному аналізу (ANOVA).*

Результати. *Середній вік для нашого населення становив 58,86 років. ANOVA демонструє значні коливання середнього показника HCD для різних регіонів: плечова кістка = 8,45±2,48, стегнова кістка = 7,09±4,06, гомілка = 8,70±2,52 та хребці = 3,69±0,73. Між віком та первинними остеонами існує сильний зворотний зв'язок. Загальна кількість фрагментів остеону зростала з віком, тоді як загальна кількість первинних остеонів та середнє значення HCD зменшувались із віком. HCA, хоча і статистично незначний, також зменшувався з віком.*

Висновок. *Отримані нами результати показують, що три гістоморфометричні параметри продемонстрували значну кореляцію з віком: фрагменти остеонів (OS-f), первинні остеони (OS-p) та HCD. Тому гістоморфометричні параметри є важливими для оцінки віку.*

КЛЮЧОВІ СЛОВА: **оцінка віку; гістоморфометричні параметри; нігерійці; криміналістика.**

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References

1. Lynnerup N, Thomsen JL, Frohlich B. Intra-and inter-observer variation in histological criteria used in age at death determination based on femoral cortical bone. Forensic Sci Int. 1998 Feb 16;91(3): 219-30.

DOI: https://doi.org/10.1016/S0379-0738(97)00197-7

2. Maat GJ, Maes A, Aarents MJ, Nagelkerke NJ. Histological age prediction from the femur in a contemporary Dutch sample. The decrease of nonremodeled bone in the anterior cortex. J Forensic Sci. 2006 Mar;51(2):230-7.

DOI: https://doi.org/10.1111/j.1556-4029.2006.00062.x

3. Kosior W. Age and notions related to it in Greek non-legal sources–the contribution to the research of Roman law. Roczniki Administracji i Prawa. 2016;16:85-96.

4. Smith HB. Standards of human tooth formation and dental age assessment. In: Kelley MA, Larson CS (eds). Advances in Dental Anthropology. New York: WileLiss; 1991. p. 143-68.

5. Lundy JK. Forensic anthropology: What bones can tell us. Lab Med. 1998;29(7):423–27

DOI: https://doi.org/10.1093/labmed/29.7.423

6. Loth SR and İsçan MY. Morphological age estimation. In: Siegal J, Saukko P, Knupfer G (eds). Encyclopedia of Forensic Sci. Academic Press; 2012 Dec 28. p.242-52.

DOI: https://doi.org/10.1006/rwfs.2000.0755

7. Cunningham C, Scheuer L, Black S. Developmental juvenile osteology. Academic press; 2016 Jul 26.

DOI: https://doi.org/10.1016/B978-012624000-9/50004-6

8. Scheuer L. Application of osteology to forensic medicine. Clin Anat. 2002 Jun;15(4):297-312.

DOI: https://doi.org/10.1002/ca.10028

9. Sobol J, Ptaszyńska-Sarosiek I, Charuta A, Oklota-Horba M, Żaba C, Niemcunowicz-Janica A. Estimation of age at death: examination of variation in cortical bone histology within the human clavicle. Folia Morphol (Warsz). 2015;74(3):378-88.

DOI: https://doi.org/10.5603/FM.2015.0021

10. Krogman WM, Iscan MY. The Human Skeleton in Forensic Medicine. 2nd Edition, Charles C. Thomas, Springfield. 1986

DOI: https://doi.org/10.1002/ajpa.1330740117

11. Buckberry JL, Chamberlain AT. Age estimation from the auricular surface of the ilium: a revised method. American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists. 2002 Nov;119(3):231-9.

DOI: https://doi.org/10.1002/ajpa.10130

12. Igarashi Y, Uesu K, Wakebe T, Kanazawa E. New method for estimation of adult skeletal age at death from the morphology of the auricular surface of the ilium. American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists. 2005 Oct;128(2):324-39.

DOI: https://doi.org/10.1002/ajpa.20081

13. Redfield A. A new aid to aging immature skeletons: development of the occipital bone. American Journal of Physical Anthropology. 1970 Sep;33(2): 207-20.

DOI: https://doi.org/10.1002/ajpa.1330330206

14. Stout SD, Dietze WH, Işcan MY, Loth SR. Estimation of age at death using cortical histomorphometry of the sternal end of the fourth rib. Journal of Forensic Science. 1994 May 1;39(3):778-84.

DOI: https://doi.org/10.1520/JFS13655J

15. Oettle AC, Steyn M. Age estimation from sternal ends of ribs by phase analysis in South African blacks. J Forensic Sci. 2000 Sep 1;45(5):1071-9.

DOI: https://doi.org/10.1520/JFS14831J

16. Keough N. Estimation of age at death from the microscopic structure of the femur (Doctoral dissertation, University of Pretoria). 2007.

17. Crowder C, Dominguez VM. Estimation of age at death using cortical bone histomorphometry. US Department of Justice, National Institute of Justice. 2013 Jan. 2013.

18. Kerley ER. The microscopic determination of age in human bone. American journal of physical anthropology. 1965 Jun;23(2):149-63.

DOI: https://doi.org/10.1002/ajpa.1330230215

19. Kerley ER. Age determination of bone fragments. Journal of Forensic Sciences. 1969 Jan 1;14(1):59-67.

20. Uytterschaut HT. Determination of skeletal age by histological methods. Zeitschrift für Morphologie und Anthropologie. 1985 Jun 1:331-40.

21. Ruddle JL. An investigation of bone histology as a potential age indicator in roe deer (Doctoral dissertation, University of London). 1997.

22. Singh IJ, Gunberg DL. Estimation of age at death in human males from quantitative histology of bone fragments. American Journal of Physical Anthropology. 1970 Nov;33(3):373-81.

DOI: https://doi.org/10.1002/ajpa.1330330311

23. Stout SD, Dietze WH, Işcan MY, Loth SR. Estimation of age at death using cortical histomorphometry of the sternal end of the fourth rib. Journal of Forensic Science. 1994 May 1;39(3):778-84.

DOI: https://doi.org/10.1520/JFS13655J

24. Maat GJR, Aarents MJ and Nagelkerke NJD. Age prediction from bone replacement: Remodeling of circumferential lamellar bone tissue in the anterior cortex of the femoral shaft of the present Dutch population. Barge's Anthropologica Leiden, Leiden University Medical Centre. 2003;10:1-19.

25. Meltem K and Dincer A. Age determination and long bone histology inStellagamastellio(Linnaeus, 1758) (Squamata: Sauria:Agamidae) populations in Turkey, SenckenbergGesellschaft fur Naturforschung. 2014;64(1):113-26.

26. De Boer HH and Maat GJR. The histology of human dry bone: A review. Barge's Anthropologica CPAG. 2003;22:49-65.

27. Thomas CD, Stein MS, Feik SA, Wark JD, Clement JG. Determination of age at death using combined morphology and histology of the femur. Journal of Anatomy. 2000 Apr;196(3):463-71.

DOi: https://doi.org/10.1046/j.1469-7580.2000.19630463.x

28. Wolf M, Streit B, Dokladal M, et al. Determining human age at death using cremated bone microctructure. Biomed J Sci& Tech Res. 2017;1(3):785-91.

DOI: https://doi.org/10.26717/BJSTR.2017.01.000304

29. Stout SD. Methods of determining age at death using bone microstructure. In: Sanders SR, Katzenberg MA (eds). Skeletal Biology of past peoples: Research Methods, Wiley-Liss. 1992. p. 21-5.

30. Ingraham MR. Histological age estimation of the midshaft clavicle using a new digital technique. M.Sc Thesis (Biology), University of North Texas, 2004.

31. Nor FM, Pastor RF, Schutkowski H. Age at death estimation from bone histology in Malaysian males. Medicine, Science and the Law. 2014 Oct; 54(4):203-8.

32. Ericksen MF. Histologic estimation of age at death using the anterior cortex of the femur. American J Physical Anthro. 1991; 84:171-79.
DOI: https://doi.org/10.1002/ajpa.1330840207
33. Steyn M, Loots M and L'Abbe EN. Adult age estimation. Forensic Anthropology, Department of Anatomy, University of Pretoria, 2004.
34. Aiello L and Molleson T. Are microscopic ageing techniques more accurate than macroscopic ageing techniques? J Archaeological Sci. 1993; 20:689-704
DOI: https://doi.org/10.1006/jasc.1993.1043
35. Frost HM. Preparation of thin undecalcified bone sections by rapid manual method. Stain Technology. 1958;33:271-76.
DOI: https://doi.org/10.3109/10520295809111862
36. Maat GJR, Robert PMV and Aarents MJ. Manual Preparation of Ground Sections for the Microscopy of Natural Bone Tissue: Update and Modification of Frost's Rapid Manual Method. Int. J. Osteoarchaeol 2001;11:366-74.
DOI: https://doi.org/10.1002/oa.578
37. Landeros O and Frost HM. Radial rate of osteon closure measured by means of tetra-cycline labelling. Henry Ford Hosp. Med. Bull. 1964;12:499-505.
38. Barer M and Jowsey J. Bone formation and resorption in normal human rib. A study of persons from 11 to 88 years of age. Clin.Orthopaedics 1967; 52:241-47
DOI: https://doi.org/10.1097/00003086-196700520-00020
39. Enlow DH. The canal system in bone. In: XXXXX (Ed), Principles of Bone Remodeling. CC Thomas, Springfield, 1963; pp. 60-74.
40. Tersigni MA. Serial Long Bone Histology: Inter- and Intra-Bone Age Estimation. PhD diss., University of Tennessee, 2005.
41. Purves S, Woodley L and Hackman L. Age determination in the Adult. In: Black S, Ferguson E (eds). Forensic Anthropology 2000-2010. 2011; Pp 29-60.
DOI: https://doi.org/10.1201/b10727-3
42. United Nations Development Programme/ Oxford Poverty & Human Development Initiative. 2019 global multidimensional poverty index. www.ng.undp.org/content/Nigeria/en/home/press-center/pressrelease/2019/new-data-challenges (2019, accessed 12 December 2019)
43. Thompson DD and Gunness-Hey M. Bone mineral-osteon analysis of Yupik-Inupiaq Skeletons. American J Physical Anthro.1981; vol. 55, pp. 1-7.
DOI: https://doi.org/10.1002/ajpa.1330550102
44. Abdullah H, Jamil MMA, Ambar R, et al. Bone histology: A key for human sex determination after death. J. Phys.: Conf. Ser. 2018; 1019: 012010 .
DOI: https://doi.org/10.1088/1742-6596/1019/1/012010

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FAVIPIRAVIR AND DEXAMETHASONE IN MANAGEMENT OF SARS-COV2 INFECTION (PILOT STUDY)

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Background. *The clinical presentation of Coronavirus disease 19 (COVID-19) varies from mild symptoms to severe illness including multiorgan dysfunction. Favipiravir is an antiviral agent which has been previously used for treatment of influenza and was recently approved for treatment of mild to moderate COVID-19 in India.*

Objective. *The Objective of this study was to assess the role of Favipiravir and Dexamethasone in patients with COVID-19.*

Methods. *A total of 17 patients were included in this observational study. The included patients were RT-PCR for SARS-Cov-2 positive with increased inflammatory markers. All patients received Antiviral therapy, Anticoagulation (Enoxaparin 0.4mg subcutaneous twice daily), Steroids (Dexamethasone 8mg daily for 5days and 4mg daily for 5 days). Viral clearance (time to RT-PCR negative), time to defervescence after antiviral therapy, time to become independent of Oxygen support was studied.*

Results. *Fever, myalgias, dry cough and dyspnea were the commonest presentation of COVID-19. All of our patients had lymphopenia. In our study 11 (64.7%) patients had bilateral ground glass opacities on CT chest while 6 had consolidation in addition to ground glass opacities. In two patients, who required non-invasive ventilation, Favipiravir was stopped and these patients received Remdesivir for a total of 5 days. In patients who received Favipiravir only, the Median time to RT-PCR negative, defervescence and oxygen independence was 8,3 and 6 days respectively.*

Conclusion. *Our observational study demonstrated improvement in the majority of patients with COVID-19 with use of Favipiravir. Additional studies are needed to compare the efficiency of Favipiravir with Remdesivir.*

KEYWORDS: **SARS-CoV-2; Favipiravir; Remdesivir; COVID-19.**

Introduction

COVID-19 or coronavirus disease 2019, is a novel illness caused by recently discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus was first identified in Wuhan, a city in the Hubei province of China in December 2019 and has since rapidly spread into a global pandemic causing considerable morbidity and mortality worldwide [1, 2]. The disease is classified as either mild to moderate disease (with no or mild symptoms up to mild pneumonia), severe disease (with hypoxia, dyspnea or >50% lung involvement on imaging within 48 hours), or critical illness (with respiratory failure, multiorgan dysfunction or shock) [3-6]. However, the vast majority (around 80 to 85%) of infections result in a mild to moderate illness [7].

The ideal therapies for management of COVID-19 are still under investigation. Favipiravir is an antiviral agent which has been previously used for treatment of influenza and was recently approved for treatment of mild to moderate COVID-19 in India [8, 9]. It has shown promise in an early non-randomized open label clinical trial in patients with non-severe disease, where use of Favipiravir was associated with faster rates of viral clearance (median time to clearance 4 versus 11 days) and more frequent radiographic improvement (in 91 versus 62 percent by day 14) compared with lopinavir-ritonavir [10]. Other robust studies are underway to assess the utility of Favipiravir in the management of COVID-19. We describe our early experience with the use of Favipiravir in patients with COVID-19.

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Methods

A total of 17 patients were included in this observational study. The included patients were RT-PCR positive for SARS-CoV-2 with increased inflammatory markers. Clinical Characteristics

of the patient population was studied. The inflammatory markers done in all patients were Interlekin-6, Ferritin, C-Reactive protein, Lactate dehydrogenase (LDH), D-Dimer. The other investigations done in all the included patients were Complete Blood count, Liver Function and Kidney Function tests, Urine examination, Blood Cultures, Serum procalcitonin. Ultrasound abdomen, Xray Chest and High-Resolution computed tomography Chest. All patients received Antiviral therapy, Anticoagulation (Enoxaparin 0.4mg SC twice daily), Steroids (Dexamethasone 8mg daily for 5days and 4mg daily for 5 days). Viral clearance (time to RT-PCR negative), time to defervescence after antiviral therapy, time to become independent of Oxygen support and development of any complication during hospital stay was studied. Patients requiring mechanical ventilation at presentation to hospital were not included in this study.

Results

Out of 17 patients, there were 7 females and 10 male patients. The mean age of patients was 43.88±14.62 and mean Body mass index (BMI) was 23.54±1.62 kg/m^2. All had high grade fever and myalgias, while 9 (52.9%) patients had dry cough on presentation. 5 patients complained of sore throat and shortness of breath was seen in 9 patients (table 1).

There were 4 patients with known Diabetes mellitus and 3 of them were hypertensive as well. Rest of the patients did not have any underlying comorbidity. However, in two patients, who developed new onset hyperglycemia during steroid course, were found to have Hba1c in Prediabetic range. Out of 17 patients, 11 (64.7%) patients had bilateral ground glass opacities on CT chest while 6 had consolidation in addition to ground glass opacities. All of our patients had Absolute Lymphocyte count (ALC) below 1500 while 4 patients had severe lymphopenia (ALC<1000 cells). The inflammatory markers and biochemical parameters are shown in table 2.

Table 1. Clinical features of patient population

Clinical feature	n (%)
Fever	17 (100)
Myalgia	17 (100)
Cough	9 (52.9)
Sore throat	5 (29.4)
Vomiting	2 (11.7)
Dyspnea	9 (52.9)
Epigastric discomfort	1 (0.05)

Table 2. Biochemical parameters and inflammatory markers

Parameter	Mean±SD
Hemoglobin (g/dL)	13.08±1.83
Platelet (lac/ µL)	3.4±0.71
Ferritin (µg/L)	615.41±307.03
ALT (IU/L)	111.5±35.7
Absolute lymphocyte count	1180±213.2
Bilirubin (mg/dL)	0.92±0.23
Triglyceride (mg/dL)	125.4±21.01
D-Dimer (ng/ µL)	566.13±207.92
LDH (U/L)	615±187.06
IL 6 (pg/µL)	80.58±98.06

In our study, 10 patients had mild transaminitis before Favipiravir and 3 developed self-limiting transaminitis after Favipiravir, 4 had normal Liver function test throughout. Two patient had acute kidney injury (AKIN class 1) that resolved during hospital stay. Blood culture was sterile in all the patients and Serum procalcitonin was negative in all the patients. Nine patients required oxygen support. Among them 7 were on oxygen support via Nasal Canula and 2 patients required non-invasive ventilation (NIV) during hospital stay. Favipiravir was given at a dose of 1800 mg twice on day 1 and subsequently 800 mg twice daily from day 2-10. In two patients, who required NIV Favipiravir was stopped, and these patients received Remdesivir for a total of 5 days. In patients who received Favipiravir only, the median time to RT-PCR negative, defervescence and oxygen independence was 8,3 and 6 days respectively. There was no thrombocytopenia, bleeding bacterial sepsis in our patient population. There was no mortality in our study population.

Discussion

The optimal management of patients with COVID-19 is rapidly evolving based on extensive ongoing research. Initial studies have suggested a clinical benefit with Remdesivir (antiviral agent) and a mortality benefit with the use of glucocorticoids. Remdesivir, a nucleotide analogue, has in vitro activity against SARS-CoV-2 [11]. Although more data from comparative, randomized trials are emerging [12, 13], available reports suggest there is likely some clinical benefit to Remdesivir prompting emergency use authorization by the FDA for severe COVID-19 in hospitalized children and adults [14]. Remdesivir needs to be administered intravenously and should be avoided in patients with transaminitis (ALT ≥5 times normal) or in

patients with an estimated glomerular filtration rate (eGFR) <30 mL/min per 1.73 m^2. It should also not be coadministered with hydroxychloroquine or chloroquine due to potential drug interactions. The use of dexamethasone (oral or intravenous) provided a mortality benefit at 28 days in hospitalised patients with COVID-19 compared to usual care alone as per a preliminary report of the RECOVERY trial, a large randomized open-label study in the United Kingdom [15]. However, no benefit was reported among patients who did not require either oxygen or ventilatory support; with a statistically non-significant trend towards higher mortality (17.8 versus 14 percent, RR 1.19, 95% CI 0.91-1.55). Also, there remain uncertainties in this preliminary report, as the baseline mortality rate in this report was higher than that from some other trials. Thus, the absolute mortality benefit in other settings may not be as high as observed in this trial. Additionally, adverse effects (including secondary infections) were not reported in the preliminary report. Another antiviral agent which is being investigated for its use in patients with COVID-19 is a purine nucleoside analogue, Favipiravir. It selectively inhibits RNA dependent RNA polymerase (RdRP), an enzyme needed for RNA viral replication within human cells, by getting incorporated instead of guanine and adenine [8]. The drug is converted into its active phosphorylated form intracellularly and subsequently is recognized as a substrate by the viral RdRP. The incorporation of a single molecule of the active form of Favipiravir terminates the elongation of viral RNA [8]. Favipiravir is known to have a broad spectrum of activity towards RNA viruses (like influenza, bunyavirus, arenavirus, flavivirus, and filoviruses causing hemorrhagic fever) including activity against oseltamivir- and zanamivir-resistant influenza viruses [8, 16]. Wang et al studied the in vitro antiviral efficiency of several drugs including Favipiravir against SARS-CoV-2, reporting that Favipiravir reduced the viral infection, albeit at higher concentrations half-maximal effective concentration (EC50) = 61.88 µM, half-cytotoxic concentration (CC50) > 400 µM, selectivity index (SI) > 6.46 [11]. Pertinently, in a previous study evaluating the efficiency of Favipiravir against Ebola virus, even with a high EC50 value in Vero E6 cells of 67 µM, the antiviral agent demonstrated 100% in vivo effectiveness in protecting mice against Ebola virus; suggesting that further clinical studies could better evaluate the in vivo response of this antiviral nucleoside [17].

Early clinical studies of Favipiravir for COVID-19 have been promising. A non-randomized open-label study by Cai et al. reported a significant reduction in time taken for viral clearance in COVID-19 patients treated with Favipiravir compared to historical controls who had received lopinavir/ritonavir [10]. This study from China involved administration of Favipiravir (1600 mg orally twice daily on day 1 followed by 600 mg orally twice daily on days 2–14) in patients with mild to moderate COVID-19. Patients ≥75 years old, those having severe or critical COVID-19, chronic liver disease or end-stage renal disease were excluded from the study. In addition to a significant reduction ($p < 0.001$) in median time to viral clearance in the Favipiravir arm (4 days; IQR = 2.5–9) compared with the historical lopinavir/ritonavir arm (11 days; IQR = 8–13), the vast majority of patients (91.4%) in the former group had radiographic improvement versus 62.2% in the latter group at 14 days. There was a significantly lower rate of adverse events in patients receiving Favipiravir (11.4% versus 55.6%; $P < 0.01$). In our study those patients who received Favipiravir only, the median time to RT-PCR negative, defervescence and oxygen independence was 8,3 and 6 days respectively. Chen et al conducted a prospective multicenter randomized open label study comparing outcomes in adult COVID-19 patients after administration of Umifenovir versus Favipiravir in addition to conventional therapy [18]. In their preprint article, they report a statistically non-significant (p=0.1396, difference of recovery rate: 0.0954; 95% CI: -0.0305 to 0.2213) higher clinical recovery rate at Day 7 in the Favipiravir group (61.2%) compared to the Umifenovir group (51.6%). Also, the Favipiravir group had significantly shorter latencies to relief in pyrexia (difference: 1.70 days, P<0.0001) and cough (difference: 1.75 days, P<0.0001). They reported only mild and manageable side effects from the use of Favipiravir. Although there is a paucity of high-grade evidence for the effectiveness of Favipiravir in COVID-19, the in vitro activity and benefits seen in early studies strongly suggest the potential for using Favipiravir in SARS-CoV-2 infection. Our observational study demonstrated improvement in the majority of patients with COVID-19 with use of Favipiravir.

Conclusions

Ease of oral administration and a profile of mild adverse effects are advantages of Favipiravir. Further evidence from well-designed

randomized controlled trials should enable clinicians to better understand the role of Favipiravir in the management of the ongoing coronavirus pandemic. Additional studies are needed to compare the efficiency of Favipiravir compared to Remdesivir.

Conflicts of Interest

Authors declare no conflict of interest.

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Author Contributions

Muzamil Latief, Obeid Shafi – conceptualization, methodology, formal analysis, writing – original draft, writing – reviewing and editing; *Muzamil Latief, Zhahid Hassan, Farhat Abbas* – data curation, writing – reviewing and editing; *Muzamil Latief, Obeid Shafi, Farhat Abbas* – investigation, formal analysis.

ФАВІПІРАВІР ТА ДЕКСАМЕТАЗОН У ЛІКУВАННІ SARS-COV2 ІНФЕКЦІЇ (пілотне дослідження)

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Вступ. *Клінічна картина коронавірусної хвороби (COVID-19) варіює від легкого перебігу до тяжких проявів з поліорганною дисфункцією. Противірусний засіб фавіпіравір, який раніше застосовувався для лікування грипу, нещодавно був схвалений для лікування COVID-19 легкого та середнього ступеня тяжкості в Індії.*

Мета. *Завданням цього дослідження – оцінка ефективності фавіпіравіру та дексаметазону при COVID-19.*

Методи. *У пілотне дослідження було включено 17 пацієнтів, у яких були позитивні ПЛР тести до SARS-CoV-2 та підвищені маркери запалення. Усі пацієнти отримували противірусну терапію, антикоагулянт (еноксапарин 0,4 мг підшкірно двічі на день), глюкокортикостероїди (дексаметазон 8 мг щодня протягом 5 днів та 4 мг щодня протягом 5 днів). Досліджувалися такі показники: тривалість перебігу хвороби до негативних результатів ПЛР тесту, час нормалізації температури на тлі противірусної терапії, швидкість відновлення самостійного дихання.*

Результати. *Лихоманка, міалгія, сухий кашель та задишка були найпоширенішими симптомами COVID-19. У всіх пацієнтів була лімфопенія. При проведенні комп'ютерної томографії грудної клітки, у 11 (64,7%) пацієнтів було знайдено симптом «матового скла», тоді як у 6 спостерігалися і симптом «матового скла» і ущільнення. У двох пацієнтів, яким була потрібна неінвазивна вентиляція, фавіпіравір було відмінено, і ці пацієнти отримували ремдесевір протягом 5 днів. У пацієнтів, які отримували лише фавіпіравір, медіана часу до негативного значення ПЛР тесту, швидкість повернення температури до нормальної та швидкість відновлення самостійного дихання становила 8,3 та 6 днів відповідно.*

Висновок. *Наше пілотне дослідження продемонструвало тенденцію до покращення у більшості пацієнтів із COVID-19 при застосуванні фавіпіравіру. Головні обмеження – мала кількість спостережень, та необхідність проведення додаткових досліджень для порівняння ефективності фавіпіравіру та ремдесевіру .*

КЛЮЧОВІ СЛОВА: **SARS-CoV-2; фавіпіравір; ремдесевір; COVID-19.**

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References

1. World Health Organization. Director-General's remarks at the media briefing on 2019-nCoV on 11 February 2020. Available at: https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020. Accessed July 28, 2020.

2. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Mar;579 (7798):265-69.

DOI: https://doi.org/10.1038/s41586-020-2008-3

3. Bajema KL, Oster AM, McGovern OL, Lindstrom S, Stenger MR, Anderson TC, et al. Persons evaluated for 2019 novel Coronavirus - United States, January 2020. MMWR Morb Mortal Wkly Rep. 2020 Feb 14;69(6):166-70.

DOI: https://doi.org/10.15585/mmwr.mm6906e1

4. Wang D, Hu B, Hu C, Zhu F, Liu X, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel Coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020 Mar 17;323(11):1061-69.

DOI: https://doi.org/10.1001/jama.2020.1585

5. Liu K, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, et al. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin Med J (Engl). 2020 May 5;133(9):1025-31.

DOI: https://doi.org/10.1097/CM9.0000000000000744

6. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020 May;8(5):475-481.

DOI: https://doi.org/10.1016/S2213-2600(20)30079-5

7. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020 Apr 7;323(13):1239-42.

DOI: https://doi.org/10.1001/jama.2020.2648

8. Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci. 2017;93(7):449-63.

DOI: https://doi.org/10.2183/pjab.93.027

9. Glenmark becomes the first pharmaceutical company in India to receive regulatory approval for oral antiviral Favipiravir, for the treatment of mild to moderate COVID-19. Glenmark Pharmaceuticals Ltd. Press release on 20 June 2020. Available at: https://www.glenmarkpharma.com/sites/default/files/Glenmark-becomes-the-first-pharmaceut-cal-company-in-India-to-receive.pdf. Accessed July 28, 2020.

10. Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental treatment with favipiravir for COVID-19: an open-label control study. Engineering (Beijing). 2020 Oct;6(10):1192-98.

DOI: https://doi.org/10.1016/j.eng.2020.03.007

11. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020 Mar;30(3):269-271

DOI: https://doi.org/10.1038/s41422-020-0282-0

12. Beigel JH, Tomashek KM, Dodd LE. Remdesivir for the Treatment of Covid-19 - Preliminary Report. Reply. N Engl J Med. 2020 Sep 3;383(10):994.

DOI: https://doi.org/10.1056/NEJMoa2007764

13. Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet. 2020 May 16;395(10236):1569-78.

DOI: https://doi.org/10.1016/S0140-6736(20)31022-9

14. Remdesivir letter of EUA. US FDA. Available at: https://www.fda.gov/media/137564/download. Accessed on: July 22, 2020.

15. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 2021 Feb 25;384(8):693-704

DOI: https://doi.org/10.1056/NEJMoa2021436

16. Shiraki K, Daikoku T. Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. Pharmacol Ther. 2020;209:107512.

DOI: https://doi.org/10.1016/j.pharmthera.2020.107512

17. Oestereich L, Lüdtke A, Wurr S, Rieger T, Muñoz-Fontela C, Günther S. Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model. Antiviral Res. 2014;105:17-21.

DOI: https://doi.org/10.1016/j.antiviral.2014.02.014

18. Chen C, Zhang Y, Huang J, et al. Favipiravir versus Arbidol for COVID-19: A Randomized Clinical Trial. medRxiv; 2020.

DOI: https://doi.org/10.1101/2020.03.17.20037432

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