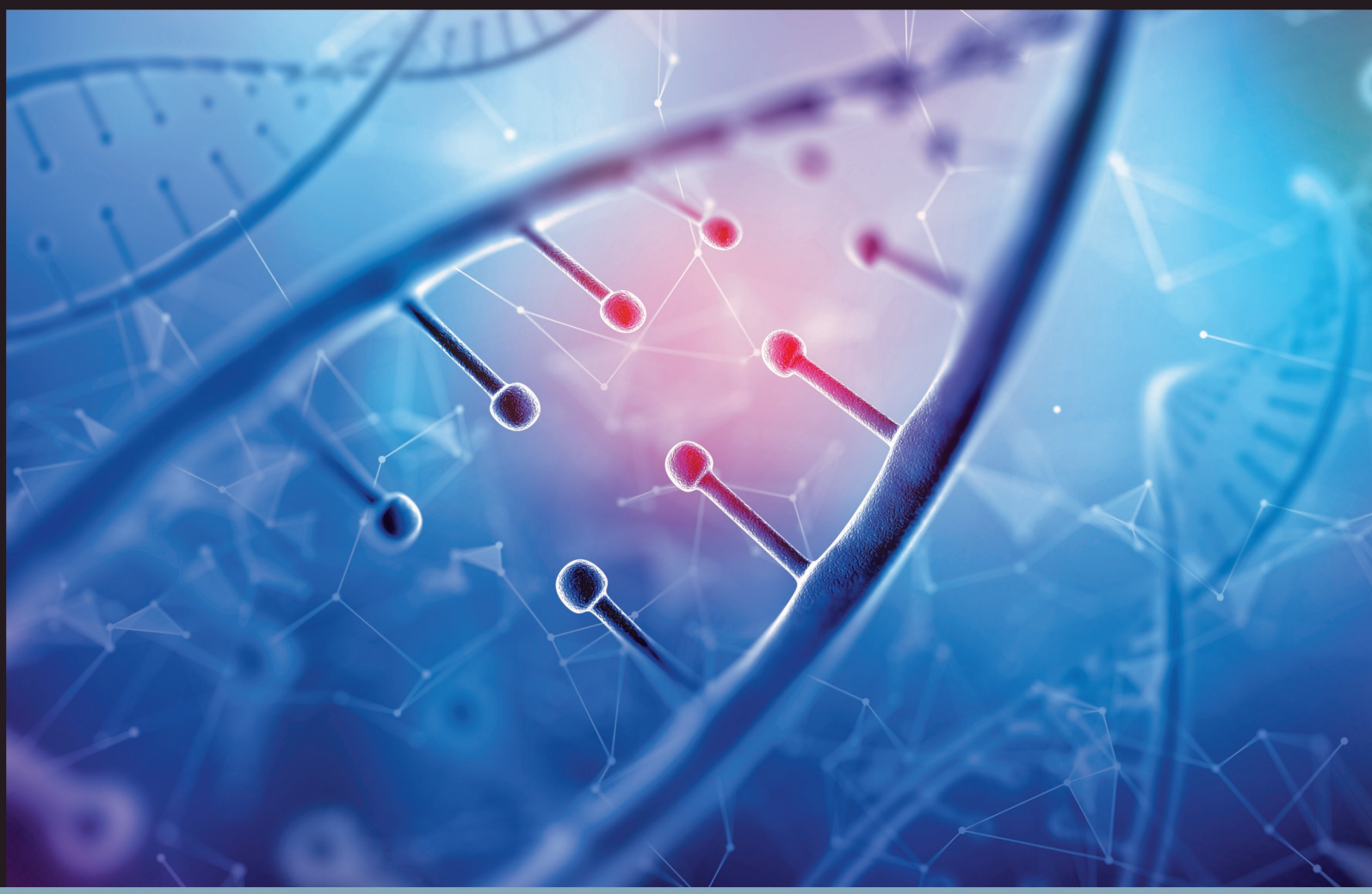
Medicine Personalized JOURNAL

Medical Journal / 8 year / No.29 / 150000Rials / 2023 Spring / ISSN 2717-3860



The Future of Medicine is Personalized

Journal Information

Name: Personalized Medicine Journal
Abbreviated Name: PMJ
Date of First Issue Published: February 2019
Concessionaire: AmitisGen TECH Dev Group
Release Period: Quarterly

Editorial Board Information

Contact Information
Tel: (98)2188985291-3
Fax: (98)2189775181
Email: Editor@pmjournal.ir
Address: No. 2, Italia Street, Tehran, Iran
Postal code : 1416673744

Journal Information

Name: Personalized Medicine Journal
Abbreviated Name: PMJ
Date of First Issue Published: February 2019
Concessionaire: AmitisGen TECH Dev Group
Release Period: Quarterly

Editorial Board Information

Contact Information
Tel: (98)2188985291-3
Fax: (98)2189775181
Email: Editor@pmjournal.ir
Address: No. 2, Italia Street, Tehran, Iran
Postal code : 1416673744

http://pmjournal.ir Oroginal Article

Spring 2023, Volume 8, Issue 29 (1-7)

Maternal Age-Related Chromosomal Aneuploidy in Human Day 3 Embryo Biopsy Blastomeres in Couples Undergoing IVF Cycles in Kuwait

Abdullah A Albahar[1*], Megdeline G Martin[2], Eman KH Bahar[3], Rana AS Sarmiti[4]

[1]Sheikh Jaber Al Ahmad Al Sabah Hospital, Kuwait.
[2]Department of Genetics, Al Soor Clinic, Kuwait.
[3]Department of Genetics, Al Soor Clinic, Kuwait.
[4]Department of Genetics, Al Soor Clinic, Kuwait.

DOI: 10.22034/pmj.2023.705457

**Corresponding author: Abdullah A Albahar, 1Sheikh Jaber Al Ahmad Al Sabah Hospital, Kuwait. Email: dab_r@live.com*

Submitted: 2023-03-25
Accepted: 2023-05-21

Keywords:
Next Generation Sequencing
Genetics
Blastomeres
Aneuploidy
Chromosomes

Abstract:
Objectives: Next Generation Sequencing (NGS) has been a popular platform for preimplantation genetic testing of embryonic aneuploidy. The main objective is to study the rate of aneuploidy in human blastomeres at day 3 embryonic division biopsy in Kuwait using NGS and to further assess the relationship between maternal age and the complexity of embryonic chromosomal aneuploidy.
Methods: Data was collected from a single genetics centre in Kuwait through electronic records. This retrospective study of 101 patient covered a period of 4 months (January 2022- April 2022). Information on chromosomal errors were stratified using contingency tables. Statistical analysis of aneuploid outcomes was carried out to estimate value between different maternal age groups. P values under 0.05 were considered statistically significant.
Result: A total of 808 blastomeres were included in the study and deemed to have met our inclusion criteria. There was a total of 572 (70.8%) aneuploid blastomeres. Highest percentage of aneuploidy was within embryos collected from maternal ages 40 years and above (72.7%). A total of 686 aberrations were within chromosomes 13, 18, 21, X, and Y, 30.3% of which were an aneuploidy within chromosome X. Majority (66.8%) of blastomeres demonstrated an aberration in at least 3 of its chromosomes and therefore deemed as complex. Overall trisomy to monosomy ratio was 3.169.
Conclusion: There was a wide-ranging variability regarding mean number of blastomeres examined across the different maternal ages. Our study has reflected the well-known association of embryonic aneuploidy with increased maternal age given the highest proportion of error (72.6%) was within blastomeres from maternal ages 40 years and above. Overall statistical analysis has however demonstrated no significant difference regarding rate and complexity of aneuploidy in blastomeres across the different maternal age groups. Larger cohort studies of aneuploidy are therefore needed for further evaluation and improve patient counselling.

INTRODUCTION

Aneuploidy refers to the presence of an abnormal number of chromosomes in a cell that traditionally takes place with the occurrence of an extra or missing chromosome (1). Aneuploidy therefore allows cells to form an unbalanced chromosome complement. Preimplantation genetic diagnosis (PGD) has been known as an effective approach for identifying aneuploidy and selecting best quality euploid embryos for transfer in patients undergoing In Vitro Fertilisation (IVF) treatment. The main principle underlying PGD testing in this circumstance is excluding embryos with abnormal chromosomes in the hopes of a better chance of a successful pregnancy (2). This mode of testing is utilised globally, particularly in cases of advanced maternal age, repeated miscarriages, and repeated implantation failure (2). Earlier methods for preimplantation genetic testing included the use of fluorescent in situ hybridisation (FISH). FISH was quick and sensitive in the identification of chromosomes at the time, nevertheless, disadvantages such as split and overlapping signals have been well documented (3). Different techniques of embryo selection were developed after years of advancements in analysis methods for preimplantation genetic testing for aneuploidy as to improve resolution and reduce

costs. Next Generation Sequencing (NGS) has been the popular platform for preimplantation genetic testing of Aneuploidy (PGT-A) since 2015, replacing the well-known comparative genomic hybridisation microarray (aCGH) and FISH (3). Development of such testing platforms and advances in laboratory practice have resulted in improvements in IVF outcomes over the past twenty years and empowered the assessment of the ploidy status of embryos before transfer (4). Age-related embryonic aneuploidy nonetheless remains as a clear issue in realising a fully successful IVF process. This association between maternal age and Aneuploidy has been firmly established in previous literatures (5,6). There, however, appears to be a lack of consensus regarding the rate of age-related aneuploidy in the State of Kuwait. This study will allow us to study this association and provide an opportunity to consider the nature of the aneuploidies identified.

OBJECTIVE

The main objective is to study the prevalence of aneuploidy in human blastomeres at day 3 embryonic division biopsy in a sample cohort in Kuwait using Next Generation Sequencing Technique (NGS). This study will also allow us to further evaluate and characterise the relationship between the nature of the human embryonic chromosomal aneuploidy and age of the female partner, including number of chromosome involvement and ratio of Trisomy to Monosomy.

MATERIALS AND METHODS

Study design and participants (patient group)

In this retrospective study, data was collected over a 4-month period (January 2022- April 2022) from a single general practice and genetics centre within Al-Ahmadi district, Kuwait. Information on 101 patients were gathered from an electronic medical database and stored in a self-composed excel sheet. Primary analysis was based on the determination of the percentage of blastomeres that were aneuploid in relation to the age of the female partner (based on age group). Information on patient details, age, number of blastomeres examined and characteristics of the chromosomal aberrations present within each blastomere was collected. Information on aneuploid rates and number of errors were stratified across the different age groups using contingency tables and statistical analysis. Out of the total 101 patients, 6 cases were excluded since the embryos examined originated from parents with a known translocation. Cased with incomplete date were also excluded from the study.

Sequencing modality and Whole Genome Amplification

Human blastomeres samples were collected from IVF centres around Kuwait. Analysis was done using Thermofisher Ion Reporter™ software 5.20. For Library Preparation, gDNA was extracted and amplified using the Reproseq™ PGS Kit. Ion SingleSeq Barcodes were added to each sample accordingly. Pooled purified library was quantified using the Qubit dsDNA HS (High Sensitivity) assay kit and the Qubit 2.0 Fluorometer to finalise the dilution of pooled purified library. With regards to template Preparation (Reproductive Ion ReproSeq Aneuploidy template), runs were created on Torrent server using TorrentSuite Software. The purified library finally loaded to IonChef Instrument. Sequencing was carried out with the Ion Gene Studio S5 Prime Instrument preceded with initialization. Analysis was successfully achieved after uploading samples data to Thermofisher Ion ReporterTM software 5.20 account for results to be analysed.

STATISTICAL ANALYSIS

Statistical analysis was carried out using Stata 17. Patient data was analysed using Fisher's exact test for comparison of data primarily categorised based on patient age, number of aneuploid embryos and nature of chromosomal aberrations present. Null hypothesis in this article claims that there was no significant difference in percentage of aneuploidy between the different age groups of the female partner. Equality of group means test (Wilks's lambda) was used to estimate value between different maternal age groups. Two sample T tests were utilised for estimation of significance of results between maternal ages under 40 and those above. *P* values under 0.05 were considered statistically significant. Analysis of outcomes and confidence intervals were constructed sing computerised standard estimates allowing us to compare degree of aneuploidy across different age groups.

ETHICS

This study was approved and registered by the centre's institutional review board in Kuwait. All patient details and data were anonymised before analysis. No informed written consent was required. This project did not require an application for Caldicott approval.

RESULTS

Participant and patient characteristics (demographic and clinical information)

95 patients in total were identified as fitting our inclusion criteria and therefore included in the study. As aforementioned, 6 cases were excluded since the embryos examined had originated from parents with a known translocation. Cases with incomplete date were also excluded from the study. All blastomeres examined were at day 3 embryonic division. A total of 808 blastomeres were included in the study. Ages

of the female partner ranged from 20 years to 49 years and divided into 5 groups. Figure 1 shows the mean, median, maximum, and minimum number of blastomeres examined according to all maternal ages included in our article. Our study demonstrated a wide ranging variability in regard to mean number of blastomeres examined across the different maternal ages with the median number exceeding 10 blastomeres only from maternal ages 28, 33, and 36 years. Most blastomeres samples were from group 4 (maternal ages 35-39 years), making about 38.4% of the total studied blastomere sample. Case demographics, results for aneuploidy and nature of chromosomal aberrations across each age group are shown in Table 1.

Euploid and Aneuploidy outcomes

There were 808 stage 3 blastomeres collected from a total of 95 patients. From the sample, there was a

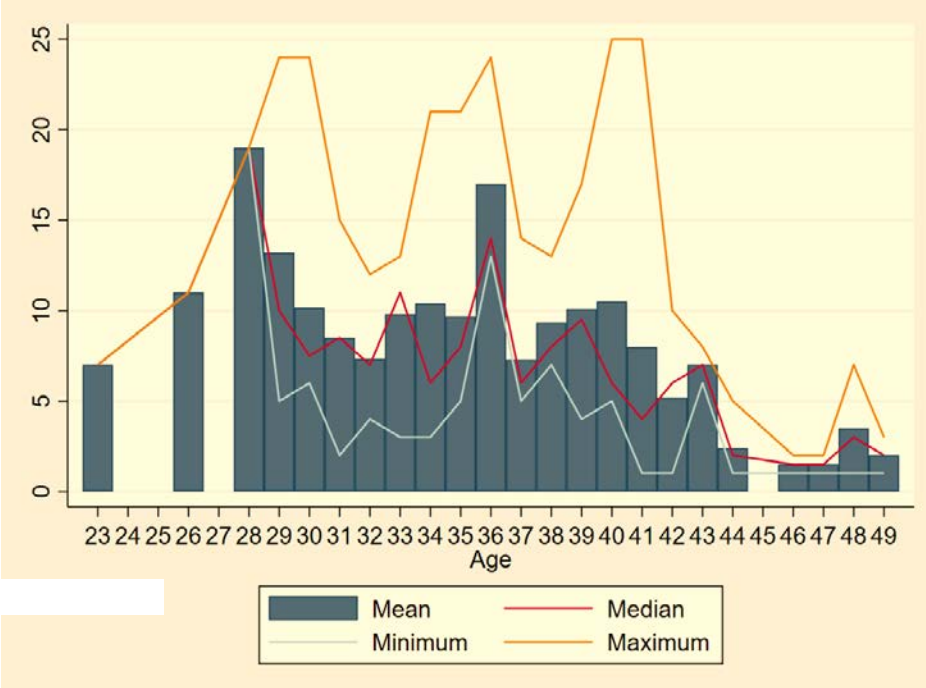


Fig1. Overall descriptive statistics of stage 3 blastomeres examined according to maternal age.

Table 1. Distribution of blastomeres, results for aneuploidy and nature of chromosomal aberration present (if any) across the different maternal age groups.

Age Grp		No of Blasto-mere	Euploid		Aneuploid		No. of Chromosomal Errors			
Group	Age Range		(n)	% of total	(n)	% of total	Single Error	Dual Error	Multiply Error (3+)	Trisomy : Monosomy
Group 1	20-24	7	2	28.5	5	71.4	0 (0%)	0 (0%)	5 (100%)	1.235
Group 2	25-29	96	27	28.1	69	71.8	13 (18.8%)	8 (11.6%)	48 (69.6%)	3.514
Group 3	30-34	223	68	30.5	155	69.5	29 (18.7%)	27 (17.4%)	99 (63.9%)	3.302
Group 4	35-39	310	92	29.6	218	70.3	44 (20.2%)	31 (14.2%)	143 (65.6%)	3.076
Group 5	40+	172	47	27.3	125	72.7	21 (16.8%)	17 (13.6%)	87 (69.6%)	3.142
Total		808	236		572		107	83		

total of 236 (29.2%) blastomeres with a fully balanced chromosomal complement and hence deemed as euploid. There was a total of 572 aneuploid blastomeres making about 70.8% of the studied population. The mean number of aneuploidies according to age of all female partners in the study is shown on figure 2. Our study showed no significant difference with the regards to aneuploidy in stage 3 blastomeres across the different age groups. P-value 0.9675. Highest rate of aneuploidy was within group 5 (those aged 40 years and above) making about 72.7% of the total number of blastomeres collected from this demographic. 155 blastomeres examined from group 3 (maternal ages 30-34 years) demonstrated a form of aberration across at least one the of the chromosomes, which approximately correlates to about 69.5% of this cohort. This group presented with the lowest fraction of aneuploidy for stage 3 blastomeres and consequently the highest proportion of euploid blastomeres within its cohort (30.5%).

Further analysis of chromosomes 13, 18, 21, X (Chromosome 23), and Y (Chromosome 24)

In this article, further analysis took place in place to study and categorise aberrations by chromosome number, more specifically chromosomes 13, 18, 21, X (Chromosome 23), and Y (Chromosome 24). Out of the 808 blastomeres, there was a total of 686 aberrations within the five chromosomes, most of which were an aneuploidy within chromosome X, forming about 30.3 % of the documented total. Least number of aberrations, irrespective of patient age, were in Chromosome Y (Chromosome 24) making 5.25% of the total number of aberrations. There was no significant difference regarding percentage of aneuploidy within those five chromosomes between the different age groups (groups 1 -5). P-value 0.9950. Further stratification and grouping of blastomeres into two age groups (maternal ages under and those equal to and above 40 years) have also shown no significant difference in proportion of aneuploidy for those 5 chromosomes between the two groups. P value 0.8322 (95% Confidence interval 0.766 -0.932 for the combined total).

Complexity of Aneuploidy and ratio of Trisomy to Monosomy

As previously mentioned, a total of 572 blastomeres exhibited an aneuploidy in at least one of its chromosomes making about 70.8% of the total number examined. Blastomeres were further characterised by complexity of the aneuploidy present, that is, either involving one, two, or three or more chromosomes. A total of 382 blastomeres demonstrated an aberration in at least 3 of its chromosomes making about 66.8%, and hence the majority, of the total sample examined. There were 107 blastomeres (18.7%) that were aneuploid for just a single chromosome within the total sample of examined blastomeres. Errors involving two chromosomes were least common, making about 14.5% of the total sample. Figure 3 further demonstrates nature and complexity of aneuploidy across the maternal ages involved in this article. Errors involving three or more chromosomes exceeded 60% of mean result in maternal ages 23, 42, 44, 46, 47, and 48 years, forming about 69.6% of the total number of chromosomal aberrations in group 5 and a 100% of that in group 1. Our study showed no significant difference between complex aneuploidy (involving three or more

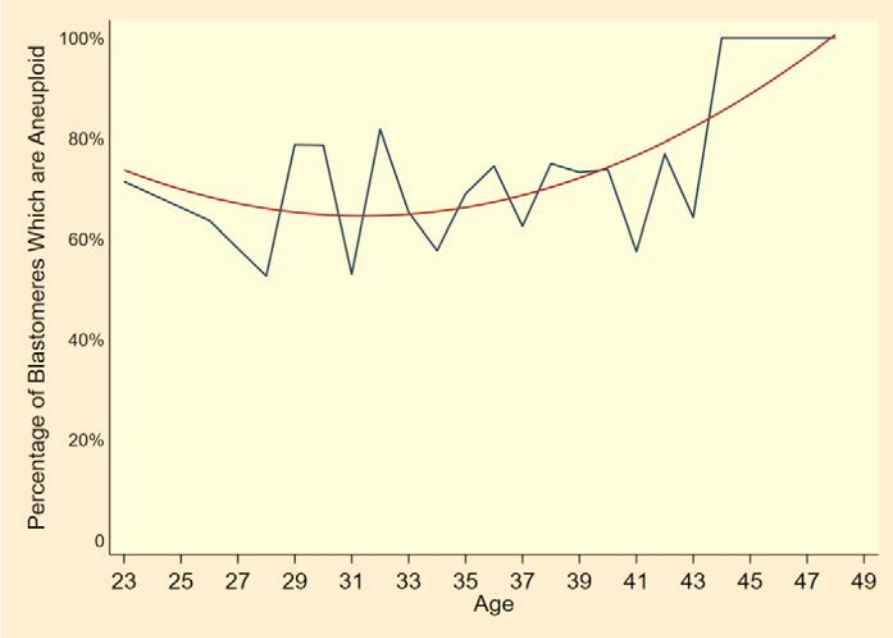


Fig2. The percentage of blastomere aneuploidy per age of female partner across the study.

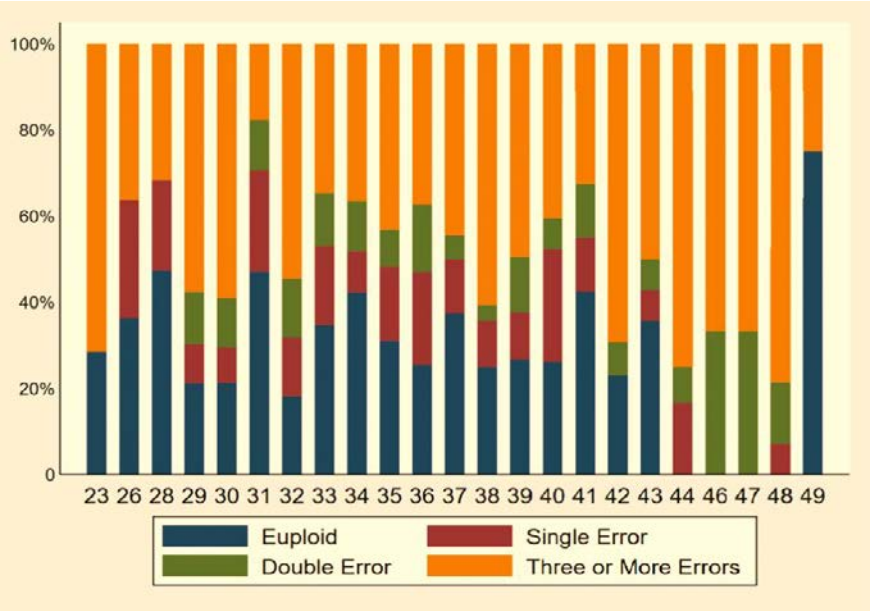


Fig3. Complexity of blastomere chromosomal aneuploidy per age of female partner involved in the study.

chromosomes) and age of female partner across all age groups (1-5). P value 0.4666. Same conclusion (no significant difference) was inferred for simpler errors involving one or two chromosomes across the different age groups.

Trisomy and Monosomy aberrations formed the two main types of errors our study. Among the 808 blastomeres examined, there were a total 3956 errors identified, 3007 were a result of Trisomy and 949 a result of Monosomy. Overall, this results in a Trisomy to Monosomy ratio of 3.169. Highest ratio of Trisomy to Monosomy errors were within group 2 (maternal ages 25-29) with a ratio of 3.514. This was a sharp contrast to the sample collected from group 1 (maternal ages 20-24) who've had a ratio of 1.235. Figure 4 illustrates the relationship between maternal age and the mean Trisomy: Monosomy ratio within the total blastomere sample.

DISCUSSION

The present study assessed estimates of aneuploidy in day 3 embryonic division pre-implanted human blastomeres using Next Generation Sequencing. With the use of NGS technique, these data represent one of

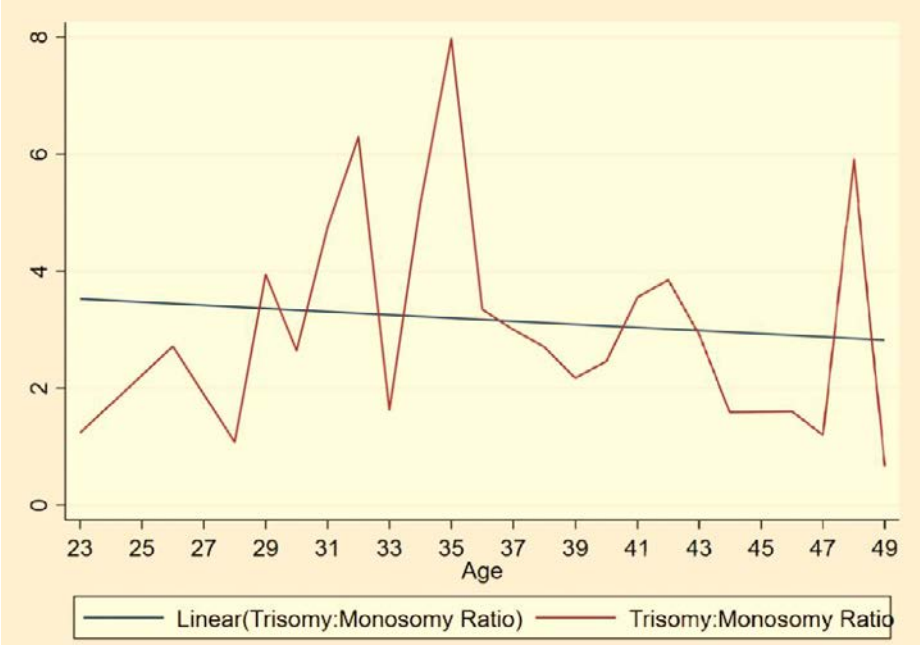


Fig4. Mean trisomy: monosomy ratio within the total blastomere sample according to maternal age.

the limited number of reports using this technology in the State of Kuwait. NGS technique has been standard worldwide, and this is attributed to its high detection rate (98%) of chromosomal aberrations (7). Despite its advantages, higher chances of reporting variants of unknown significance have also been well recognised (3). Overall, 572 (70.8%) of the blastomeres in our studied sample demonstrated an abnormal chromosome complement and hence deemed as aneuploid regardless of maternal age or complexity of the aberration present. We found this result to be in keeping with previous literature and consensus with established estimates of aneuploidy in human IVF embryos documented to be within 50-80-% (8).

This study was also able to explore the relationship between aneuploidy and maternal age in the general IVF population in Kuwait. It has been well documented that a high incidence of chromosomal aneuploidy occurs with maternal ages 35 years and older (7). This association has been known to complicate pregnancies, with chromosomal abnormalities (majority of which were aneuploidy of the embryo) being identified in approximately 50% of first trimester miscarriages (9). Evaluation of the presence of such association has been partially reflected by in our study given the highest percentage of aneuploidy was within blastomeres from maternal ages 40 years and above, making about 72.6% of embryos from this demographic. Overall evaluation of aneuploidy from previous research have not only established an increase in its frequency with maternal age but also demonstrated an increase in

proportion of embryos with more than one aneuploid chromosome (4). Our study has shown no significant difference regarding the complexity of chromosomal aberration within the blastomeres and age of female partner across all age groups. This could partly be due to the limited population sample obtained in the period our study covered, and therefore no such relationship could be inferred. Moreover, when evaluating the trisomy to monosomy ratio of the blastomeres, it was clear that group 1 (youngest maternal age) have demonstrated the lowest trisomy: monosomy ratio (1.235). This finding was in accordance with previous literatures that associated higher ratios with increased maternal ages (4).

Our study was primarily based on the examination of day 3 embryonic division blastomeres from parents with no known chromosomal translocations. Many techniques, including NGS have been employed for PGD at blastocyst stage, which is equivalent to an embryo at day 5 embryonic division, however, only a limited number of studies have reported outcomes on day 3 embryonic division blastomeres (2). We found that equal number of embryos are available at day 3 embryonic division and day 5 embryonic division, and biopsies (in both days) do not adversely affect embryo viability, chromosome configuration and ultrastructure (10, 11). Nevertheless, biopsy at day 3 embryonic division may yet cause an inaccurate assessment of the potential development of a euploid embryo because of the high mosaicism at this stage of development (12). Other technical limiting factors that may play a role in the outcomes and must be acknowledged, are included; timing of embryo biopsy, vitrification, and transfer during IVF procedure.

The limitation of our study was the size of the population. Therefore, larger cohort studies of aneuploidy for further evaluation, increase generalisability, and improve patient counsellings are needed.

Data sharing and availability statement

The data that supports the findings in this study are available from the corresponding author upon reasonable request.

Acknowledgment

No funding resources to declare.

Conflict of interest:

The authors have no conflict of interest with respect to the research, authorship, and publication of this article.

Author contributions:

Dr Abdullah A Albahar designed the work and study. Rana AS Sarmiti and Megdeline G Martin have acquired and collected the data. Dr Abdullah A Albahar have analysed and interpreted the data. Dr Abdullah A Albahar and Megdeline G Martin drafted this article. All authors revised the final manuscript and agreed to be accountable for all aspects of the work. Final approval of the version to be published by Dr Abdullah A Albahar Megdeline G Martin· Eman KH Bahar and Rana AS Sarmiti.

REFERENCES

1.F. GAJ, Doebley JF, Peichel CL, Wassarman DA. Introduction to genetic analysis. 12th ed. New York, NY: W.H. Freeman & Company/Macmillan Learning; 2020.

2.Ye Y, Ma J, Cui L, Lu S, Jin F. A rapid NGS-based preimplantation genetic testing for chromosomal abnormalities in day-3 blastomere biopsy allows embryo transfer within the same treatment cycle. Frontiers in Genetics. 2021;12.

3.Piyamongkol, W. Pre-Implantation Genetic Testing for Aneuploidy (PGT-A). Thai J Obstet Gynaecol. 2020; 28, 130-135.

4.Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, et al. The nature of aneuploidy with increasing age of the female partner: A review of 15,169 consecutive trophectoderm biopsies

evaluated with comprehensive chromosomal screening. Fertility and Sterility. 2014;101(3). doi:10.1016/j.fertnstert.2013.11.004.

5.Hassold T, Hunt P. To err (meiotically) is human: The genesis of human aneuploidy. Nature Reviews Genetics. 2001;2(4):280–91.

6.Chiang T, Schultz RM, Lampson MA. Meiotic origins of maternal age-related Aneuploidy1. Biology of Reproduction. 2012;86(1). doi:10.1095/biolreprod.111.094367.

7.Alyafee Y, Alam Q, Tuwaijri AA, Umair M, Haddad S, Alharbi M, et al. Next-generation sequencing-based pre-implantation genetic testing for aneuploidy (PGT-A): First report from Saudi Arabia. Genes. 2021;12(4):461.

8.Daughtry BL, Rosenkrantz JL, Lazar NH, Fei SS, Redmayne N, Torkenczy KA, et al. Single-cell sequencing of primate preimplantation embryos reveals chromosome elimination via cellular fragmentation and blastomere exclusion. Genome Research. 2019;29(3):367–82.

9.Blyth U, Craciunas L, Hudson G, Choudhary M. Maternal germline factors associated with aneuploid pregnancy loss: A systematic review. Human Reproduction Update. 2021;27(5):866–84.

10.Chatzimeletiou K, Petrogiannis N, Sioga A, Emmanouil-Nikoloussi E-N, Panagiotidis Y, Prapa M, et al. The human embryo following biopsy on day 5 versus day 3: Viability, ultrastructure and spindle/chromosome configurations. Reproductive BioMedicine Online. 2022;45(2):219–33.

11.Demko ZP, Simon AL, McCoy RC, Petrov DA, Rabinowitz M. Effects of maternal age on euploidy rates in a large cohort of embryos analyzed with 24-chromosome single-nucleotide polymorphism–based preimplantation genetic screening. Fertility and Sterility. 2016;105(5):1307–13.

12.Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. New England Journal of Medicine. 2015;373(21):2089–90.

http://pmjournal.ir Reviwe Article

Spring 2023, Volume 8, Issue 29 (8-13)

Personalized Medicine and Health Promotion: the Gut Microbiome's Key Function

Tahereh Mehdizadeh[1]*, Jafar Jafari[2]

[1]Department of biomedical engineering, Faculty of engineering, Maziar University, Royan, Iran.
[2]Build Goal Health, Inc Ceo & Founder, Los Angles, California, United States.

**Corresponding author: Tahereh Mehdizadeh, Department of biomedical engineering, Faculty of engineering, Maziar university, Royan, Iran. Email: tahere_mehdizade@yahoo.com.*

DOI: 10.22034/pmj.2023.705458

Submitted: 2023-03-22
Accepted: 2023-05-24

Keywords:
Personalized Medicine
Microbiome
Health
Therapeutic strategies

Abstract:

It has been known for quite some time that gut microbiota plays an important role in human health and illness. Recent years have seen a surge in interest in the human gut microbiota, and the advent of metagenomic investigations has greatly aided our understanding of the resident species and their potential uses. The human digestive tract is home to billions of bacteria, making up the varied gut microbiota. At birth, the gut microbiome begins to take shape and proliferate, and throughout life, numerous genetic, dietary, and environmental variables will shape and multiply this community. Alterations to the gut microbiota's structure and function may affect digestion, metabolism, and the immune system. Meanwhile, personalized medicine, a new therapeutic approach, has opened a new door in the medical sciences, and the link between the microbiome and personalized medicine is one of the most intriguing areas of study going forward. Since the link between this two axis is new, there are few research on it. Therefore, in this review study, the relationship between the gut microbiome, drug interactions, disease progression, and personalized medicine has been discussed.

INTRODUCTION

Biomedical research has mostly focused on identifying and targeting disease-associated pathways to develop pathway-targeted therapies. In multi-factorial disorders, this approach ignores inter-individual diversity in disease emergence and treatment outcome (1). Individual-specific data and its impact on human physiology have been disclosed by the genetic a uprising allowing personalized or precision medicines. Oncology research over the last decade has enabled human genome screening to reveal a range of germ-line encoded mutations, permitting individual-specific preventative and treatment options. Precision medicine has enabled patient categorization based on therapy response and adverse events, as well as genetic contribution to illness etiology (2). The microbiome has recently gained recognition as a key factor to the wellness of humans, and we highlight why the microbiome is an essential component of the precision medicine project in the present article (3).

The microbiome is a complex network of microorganisms that reside inside and on the human body, including their genes and collective activities. A healthy microbiome has a set of shared traits that distinguishes it from non-healthy people; hence, knowing the microbiome's distinctive qualities may aid in the detection and identification of disease-associated microbiomes (4). The healthy person's microbiome is very varied, with a significant amount of beneficial microbes that can withstand the changes that occur during each period of physiological stress; whereas the disease-associated microbiota is less broad, with a lower number of beneficial bacteria that leads to disease in the presence of infection (5). The key issue that researchers are working on is understanding the possible features of microbiome diversity among people. Traditional techniques, such as culture, have produced very little information in this area, but modern approaches, such as NGS, have introduced an adequate knowledge of this population and their combinations, as well as identified the archaea, bacteria, and viruses in the body (6). Disturbance in microbial ecology has been linked to a variety of disorders, including diabetes and inflammatory bowel disease; the human microbiome may be utilized as a major diagnostic indicator, and researchers are also focusing on its therapeutic significance. Although the microbiome is an interesting target for generating personalized

treatment methods, finding strategies for developing reliable and reproducible microbiome-based detection and treatment solutions remains a challenge (7). The scientific community's significant effort, along with its partnership with fast-rising biotech businesses, provides a positive prognosis for creating microbiome-dependent and microbiome-targeted diagnostics and therapies (7).

Co-metabolism between microbiota and the host in drug metabolism and toxicity

The direct and indirect interactions between gut microbiota and xenobiotics on medication pharmacological and toxic effects may cause drug failure and patient mortality. Thus, precision medicine requires an understanding of how gut microbiota affects medication effectiveness and toxicity (8). We examine how gut microbiota affects therapeutic effectiveness and toxicity and the processes. Humans spend their whole lives surrounded by essential microorganisms, which alter their capacity to detoxify xenobiotics (including medications and food substances) through microbiota-host co-metabolism. Phase I and phase II reactions are part of the host detoxification systems (8). Phase I metabolism, which includes oxidation, reduction, and hydroxylation, is primarily mediated by cytochrome P450 (CYP) enzymes in the liver, stomach, and other tissues to improve xenobiotic excretion in urine by enhancing the polarity of foreign substances. The conjugation process, which includes glucuronidation and sulfonation, is part of phase II metabolism (9). To promote urine excretion, foreign chemicals are conjugated with endogenous molecules via host enzyme transfer mechanisms. Sulfotransferase (SULT), uridine 5'-diphospho-glucuronosyltransferase/UDP-glucuronosyltransferase (UGT), N-acetyltransferase (NAT), and glutathione S-transferase (GST) are among the phase II enzymes. Over 70% of the top 200 medications given are processed in the liver, while approximately 25% are removed in the kidney11, and approximately 50% of pharmaceuticals are metabolized through the P450 enzyme system, underscoring the importance of the P450 enzyme systems in drug metabolism (9). In addition to the host drug metabolism system, the gastrointestinal microbiota plays significant roles in drug metabolism via the release of microbial drug-metabolizing enzymes and microbiota-host co-metabolism (10). While the effects of intestinal microbiota on drug metabolism have been studied for many years, only about 40 drugs and natural products have been thoroughly examined to date. The gut microbiota usually modulates the oral drug bioavailability or half-life by altering the capacity of drug-metabolizing enzymes or expression of genes involved in drug metabolism in host tissues. The oral drug bioavailability or half-life by altering the capacity of drug-metabolizing enzymes or expression of genes involved in drug metabolism in host tissues (11). Furthermore, since many illnesses are connected with gut dysbiosis or vice versa, the individual composition or function of gut microbiota is likely to be impacted by environmental variables such as foods and antibiotics, or the host's physiological condition. Individual differences in response to drug therapy are associated with differences in gut microbiota (11). For example, digoxin, an originally isolated cardiac glycoside from foxglove plants, is a typical example of a substance that can be inactivated by intestinal microbiota. Utilizing digoxin in a clinical setting is challenging due to the drug's narrow therapeutic range. In about 10% of patients, a significant quantity of digoxin is converted into dihydrodigoxin, a cardio-inactive metabolite. The conversion of digoxin by gastrointestinal microbiota in patients can account for the inactivation of more than fifty percent of the administered digoxin, which substantially affects the drug's bioavailability and clinical toxicity (12).

The Role of the gut microbiota in drug-drug interactions

Drug-drug interactions may also be attributed to gut microbiota. Antimicrobial drug resistance has become a severe worldwide health concern, contributing to a rise in infection-related deaths. In the clinic, the use of antibiotic combinations is promoted to treat multidrug-resistant bacterial infections; however, their effects on microbiota are unknown (13). Profiling of roughly 3,000 antibiotic, human-targeted medication, and food additive combinations revealed that 70% of drug-drug interactions are species-specific, 20% are strain-specific, and antagonism is more prevalent than synergism. Aside from antibiotics, the most notable example of medication-drug interactions involving gut microbiota is the co-administration of the antiviral medicine sorivudine with the anticancer drug 5-fluorouracil (5-FU) or 5-FU prodrugs, which has resulted in fatalities and severe adverse effects in Japan. Sorivudine may be converted by gut bacteria into (E)-5-(2-bromovinyl) uracil (BVU). BVU inhibits liver dihydropyrimidine dehydrogenase, a catabolic enzyme responsible for 5-FU detoxification. Thus, combining sorivudine and 5-FU raises circulation 5-FU levels, resulting in 5-FU-associated mortality (14).

The function of microbiome metabolites in disease progression

With the emergence of sophisticated illnesses such as cancer, the relationship between environmental, microbiome, and cancer consequences may become quite complex. Cancer is indicated by changes in cell metabolism and inflammation (15). Even if host-microbiome reactions to cancer are not viewed as a necessary event, the presence of microbial compounds

in certain malignancies, such as colorectal cancer (CRC), can be indirectly significant. Cancer cells communicate with bacterial quorum sensing peptides (QSP) according to in vitro research (16). Bacillus-derived QSPs can induce invasive tumor cells in a process called Epithelial mesenchymal-like (EMT-Like) (involved in CRC metastasis) when the bacteria are under duress. Under these circumstances, QSPs engage in both metastatic and angiogenesis activities. In some kinds of cancer, microbial activity may limit treatment efficiency or influence tumor formation. Lifestyle and food are other important factors in shaping the microbiome (17). Furthermore, the creation of numerous metabolites by gut microbiota is efficient in cancer-promoting and cancer-protective induction; however, distinct drivers remain unknown. Microbiome-derived metabolites have been shown to have the ability to contribute to cancer development (18). Food is a rich source of these metabolites; for example, high-fat and high-protein meals are common in the current Western diet, which is one of the risk factors for cancer incidence. Bile acid (BA), on the other hand, is a signaling molecule related to metabolic balance. Specific enzymes convert BA to SBA, which may be carcinogenic (19). In vitro, investigations have demonstrated that 1 hour of exposure to SBA compounds such as deoxycholic acid (DCA) and lithocholic acid (LCA) causes substantial DNA damage with a dose-dependent behavior. According to studies, the African-American population had a higher incidence and mortality rate from CRC than the Native American population (19). The microbiomes of these two groups (African-American and Native American) were analyzed, and the African-American group was plentiful in Bacteroides species, while the Native American group was abundant in Prevotella species. Furthermore, the encoded genes for SBA and fecal SBA in the first group had higher levels, whereas short-chain fatty acids were higher in Native Americans, and thus studies reported that phenotypic and developmental differences of a specific disease are possible, and these differences are primarily due to different diets and microbiome combinations (20). Saccharolytic fermentation is induced by fiber-rich diets owing to several kinds of gut microorganisms that create short-chain fatty acids, notably acetate, propionate, and butyrate (21). Bacteroidetes, for example, contain high quantities of acetate and propionate, while Firmhicute bacteria have high levels of butyrate. Butyrate is linked to some anti-cancer actions. Butyrate, for example, may promote S-phase ablation in colorectal cancer cells, resulting in growth suppression through apoptosis and the production of cell regulators such as P21 and cyclin B1. Interestingly, the butyrate effects are cell-dependent; in normal cells, butyrate increases proliferation as a source of energy, but in cell lines, butyrate suppresses proliferation and produces death (21).

The microbiome's involvement in precise diagnosis and personalized therapy

Precision medicine and personalized nutrition are now recognized as potential techniques for improving health outcomes by adapting treatment tactics and dietary regimens to a person's unique genetic, environmental, and lifestyle characteristics (22). These methods seek to shift away from the conventional one-size-fits-all approach to health care and nutrition in favor of offering individualized treatment that is suited to the individual's particular requirements. The function of the microbiome in improving health is a prominent topic of research in this discipline. The process of detecting and describing an individual's unique microbial profile, known as microbiome analysis, may give important insights into an individual's health condition and be utilized to build individualized therapies. Microbiome analysis, for example, has been used to identify particular microorganisms related to various health disorders such as inflammatory bowel disease and type 2 diabetes. This data may be utilized to create targeted therapies based on the individual's unique microbial profile (22).

Various data show that disruption of the microbiota-host interaction is linked to a variety of disorders, including IBD, diabetes, cirrhosis, and colorectal cancer (23). Recently, studies on the interactions between bacteria and cancer treatment drugs have been conducted, and the findings suggest that interactions of the bacteria mediated by the immune system are required for drug efficacy, even though little information is available on the effects of human microbiome combinations and treatment outcomes in cancer patients (24). Many studies have indicated that patients can react to or not respond to immunotherapy based on gut microbiota combinations, which may be taken into account when evaluating medication interactions. Furthermore, the importance of the gut microbiome as a biomarker for illness phenotype, prognosis, and treatment response is extensively established in connection to the modification of microbial population structure in diverse diseases (25). Microbiome analysis sheds light on the intricate relationships that exist between microbial populations and human health, as well as the function of the microbiome in disease genesis and progression. Microbiome analysis, for instance, has been used to discover distinct microbial communities linked to illness states, such as the depletion of certain bacterial species in individuals with inflammatory bowel disease (26). This knowledge may be utilized to create novel diagnostic tools and medicines that target the microbiome, eventually enhancing disease control and patient outcomes. A

better knowledge of the components that contribute to illness genesis and progression may be gained by microbiome study. Microbiome analysis drives the development of novel diagnostic tools and therapies that target the microbiome by finding distinct microbial populations that are related to disease states. For example, fecal microbiota transplantation (FMT) is a microbiome-based treatment that is beneficial in treating recurrent Clostridium difficile infection, a disease that is often resistant to standard antibiotic regimens (27). FMT includes the transfer of fecal matter from a healthy donor into the patient's gastrointestinal tract to restore a healthy microbiome composition and function. FMT has been found in studies to be very successful in treating recurrent Clostridium difficile infection, with cure rates as high as 90%9-11. Microbiome analysis, in addition to FMT, informs the development of additional microbiome-based medicines (27). Probiotics, prebiotics, and synbiotics are all examples of treatments that try to change the makeup and function of the microbiome. Microbiome analysis can be utilized to determine specific microbial communities associated with disease states and to select probiotic strains that can restore a healthy microbiome composition and function. Similarly, prebiotics and synbiotics can be utilized for boosting the growth of beneficial microbial communities and recover a healthy microbiome structure and function (27).

Other intriguing findings revealed that Akkermansia, Faecalibacterium, and Bifidobacterium strains are connected with anti-inflammatory reactions, which is an immune system arm that inhibits over-response activation and leads to the development and maintenance of homeostasis. For example, the relative fall of A. muciniphila in the gut has been linked to a variety of disorders, including IBD, type II diabetes, and others. Similarly, F. prausnitzii reduces intestinal inflammation caused by the generation of particular metabolites generated from host cells or bacteria in the intestines and peripheral circulation (28). All of these researches indicate that precision medicine, including the gut microbiome, has therapeutic promise.

Improving health by targeting the microbiome

The microbiome's appealing feature is its flexibility and ability to change portions of the microbiome, in addition to acting as detecting and therapeutic biomarkers and influencing therapeutic responses to drugs (29). Antibiotics have typically been employed to target microbial populations, that are necessary as well as efficient for managing systemic diseases induced by invasions of pathogens. However, the negative impacts on microbial community structure alongside human side consequences make targeting the microbiome as precision medicine less appealing. In addition, there is still a need to develop pathogen-targeted medicines by identifying specific targets that limit the antibiotic's reach. A novel technique includes mining the microbiota for potential therapies through recognizing specific behaviors that influence the host, allowing us to modify the microbial community's functioning without harming the microbial population itself. One example is the role of tri-methyl amine oxidase (TMAO) in atherosclerosis, as well as the inhibition of microbe TMA lyases by 3, 3-dimethyl-1-butanol (DMB), which lowers bacterium TMA production in an excessive choline diet-fed mice animal. Even though precise route focusing on, DMB generated microbiome modifications, demonstrating the complexities of microbial relationships within these natural environments. Probiotics, prebiotics, and nutritional therapy are among more ways to address the microbiome (31, 32).

Early probiotics (living microorganisms that provide health advantages to the human body when eaten in proper amounts) were dominated by organisms of the genera Lactobacillus and Bifidobacteria, but they lacked precision in terms of addressing a purpose in biology (33). In comparison to a placebo, a recent comprehensive review of medium to superior controlled research using probiotics indicated no major impact on gut flora. The clinical effectiveness of present-day probiotics is hard to evaluate because of small sample sizes hindering power, variation in bacteria strains used, endpoints, treatment duration, and molecular methods for studying the gut microbiota, storing of initial parameters like eating habits, and the absence of robust preliminary mechanistic information (33).

Because diet is the major source of nutrition for organisms, it has a considerable impact on the microbiome. Dietary interventions may be divided into three groups. Using microbiome markers to improve dietary interventions, customizing the diet depending on the microbiome, and changing the microbiota via nutrition are all examples of how to use microbiome indicators to optimize dietary interventions. Dietary therapy that lower fermentable oligo-, di-, monosaccharides, and polyols (FODMAP) have been shown to aid persons with IBS (34). Continuous use of such a measure, on the other hand, may diminish microbial short-chain fatty acid production, which might be harmful to individuals.

New research revealed microbial indicators that indicate a good response to FODMAP72, which has the potential to enable therapeutic optimization and the reduction of unwanted side effects in patients who are less likely to react. Considering the importance of the microbiome in dietary metabolism of nutrients, one critical function of the intestinal microbiome is its influence on host reactions to food components (35). In an excellent investigation of 800 participants, Zeevi et al. discovered considerable interpersonal

variability in post-prandial glycemic reactions to dietary components. They observed that incorporating microbiome-derived characteristics significantly improved the accuracy of the prediction of glycemic reactions in the forecast engine used to create these forecasts. It is worth mentioning that modifications in the intestinal microbiota in response to a comparable dietary change may vary based on a person's microbiome (35). Considered together, it is obvious that, while the relationship between food and the gut microbiota is multifaceted, it is critical in defining host responses to diet and anticipating alterations in the microbiome in the face of diets.

CONCLUSION

In this work, we underline the importance of integrating the gut microbiome as a part of customized or precision medicine to improve detection, reduce illness danger, and increase early identification and treatment. Microbial fingerprints have the potential to be precise, non-invasive, accessible, and cost-effective tools for phenotyping, degree of severity, and prediction in disease diagnosis. Because the microbiome is involved in the metabolism of several chemical compounds, it is a key component in affecting medicine accessibility, efficacy, and toxic effects, making it necessary for devising personalized therapy regimens.

Last but not least, the ability to alter the microbiome makes it attractive for developing personalized therapeutic approaches through precise microbiome targeting. Treatments for multi-factorial diseases including inflammatory bowel disease, obesity, and type 2 diabetes may be developed using strategies that target specific microbial pathways appropriate to a person's microbiome. The next frontier in the field of customized healthcare will be the development of precision probiotics through genetic engineering, next-generation prebiotics as a result of a greater understanding of metabolism interactions among members of the microbial environment, and personalized diets tailored to a the individual's microbiota. The future is generally quite positive, but there are also considerable challenges. We must create standardized collecting, sequencing, and analysis protocols that improve the repeatability of data across centers and reduce biases in their interpretation if we are to apply microbiome-based diagnostics and therapies. The bulk of present research is focused on illness association; however, in order to generate more precise biomarkers, we need to better understand the processes by which the microbiota affects many aspects of human disease. The function of other microorganisms like fungi, bacteriophages, and parasites is also still poorly understood, as is the communication between different kingdoms of microbes and the host. We will develop more thorough techniques to counter the effects of the microbiome on the host as we elucidate different facets of these complex relationships. Despite these challenges, a key element of the future age of patient care will be the integration of microbiome-based detection and therapies with other aspects of customized healthcare, such as Pharmaceutical Genomics and epigenomics. The proper patient will receive the right therapy thanks to this integration, which will also help to cut down on negative side effects and medical expenses.

REFERENCES

1. Feng, W., Liu, J., Ao, H., Yue, S., & Peng, C. Targeting gut microbiota for precision medicine: Focusing on the efficacy and toxicity of drugs. Theranostics. 2020 ; 10(24), 11278–11301.
2. Budnitz, D. S., Lovegrove, M. C., & Crosby, A. E. Emergency department visits for overdoses of acetaminophen-containing products. American journal of preventive medicine. 2011 ; 40(6), 585-592.
3. Behrouzi, A., Nafari, A. H., & Siadat, S. D. The significance of microbiome in personalized medicine. Clinical and translational medicine. 2019 ; 8, 1-9.
4. Hisada, T., Endoh, K., & Kuriki, K. Inter-and intra-individual variations in seasonal and daily stabilities of the human gut microbiota in Japanese. Archives of microbiology. 2015 ; 197, 919-934.
5. Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., ... & Wang, J. A human gut microbial gene catalogue established by metagenomic sequencing. nature. 2010 ; 464(7285), 59-65.
6. Scheline, R. R. Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacological reviews. 1973 ; 25(4), 451-523.
7. Amedei, A., & Boem, F. I've gut a feeling: microbiota impacting the conceptual and experimental perspectives of personalized medicine. International journal of molecular sciences. 2018 ; 19(12), 3756.
8. Li, H., He, J., & Jia, W. The influence of gut microbiota on drug metabolism and toxicity. Expert opinion on drug metabolism & toxicology. 2016 ; 12(1), 31-40.
9. Di, L. The role of drug metabolizing enzymes in clearance. Expert opinion on drug metabolism & toxicology. 2014 ; 10(3), 379-393.
10. Cho, I., & Blaser, M. J. The human microbiome: at the interface of health and disease. Nature Reviews Genetics. 2012 ; 13(4), 260-270.
11. Li, H., & Jia, W. Cometabolism of microbes and host: implications for drug metabolism and drug-induced toxicity. Clinical Pharmacology & Therapeutics. 2013 ; 94(5), 574-581.
12. Currò, D.The role of gut microbiota in the modulation of drug action: a focus on some clinically significant issues. Expert Review of Clinical Pharmacology. 2018; 11(2), 171-183.
13. Brochado, A. R., Telzerow, A., Bobonis, J., Banzhaf, M., Mateus, A., Selkrig, J., ... & Typas, A. Species-specific activity of antibacterial drug combinations. Nature. 2018 ; 559(7713), 259-263.
14. Nakayama, H., Kinouchi, T., Kataoka, K., Akimoto, S., Matsuda, Y., & Ohnishi, Y. Intestinal anaerobic

bacteria hydrolyse sorivudine, producing the high blood concentration of 5-(E)-(2-bromovinyl) uracil that increases the level and toxicity of 5-fluorouracil. Pharmacogenetics. 1997 ; 7(1), 35-43.
15.Hanahan, D.Hallmarks of cancer: A 2012 perspective. Annals of oncology, 23, ix23. 2012.
16.Contreras, A. V., Cocom-Chan, B., Hernandez-Montes, G., Portillo-Bobadilla, T., & Resendis-Antonio, O. Host-microbiome interaction and cancer: potential application in precision medicine. Frontiers in physiology. 2016 ; 7, 606.
17.Debunne, N., Wynendaele, E., Janssens, Y., De Spiegeleer, A., Verbeke, F., Tack, L., ... & De Spiegeleer, B. The quorum sensing peptide EntF* promotes colorectal cancer metastasis in mice: a new factor in the microbiome-host interaction. BioRxiv. 2020 ; 2020-09.
18.Kong, C., Liang, L., Liu, G., Du, L., Yang, Y., Liu, J., ... & Ma, Y. Integrated metagenomic and metabolomic analysis reveals distinct gut-microbiome-derived phenotypes in early-onset colorectal cancer. Gut. 2023; 72(6), 1129-1142.
19.Marion, S. A. M. Biogeography and biochemistry of bile acid 7-dehydroxylation in the mammalian gut (No. THESIS). EPFL. 2020.
20.Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... & Glöckner, F. O. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic acids research. 2012; 41(D1), D590-D596.
21.Portincasa, P., Bonfrate, L., Vacca, M., De Angelis, M., Farella, I., Lanza, E., ... & Di Ciaula, A. Gut microbiota and short chain fatty acids: implications in glucose homeostasis. International journal of molecular sciences. 2022 ; 23(3), 1105.
22.Aderinto, N., & Olatunji, G. D. The role of microbiome analysis in precision medicine and personalized nutrition for improving health outcomes in Africa: a correspondence. IJS Global Health. 2023 ; 6(3), e137.
23.Li, X., Guo, J., Ji, K., & Zhang, P. Bamboo shoot fiber prevents obesity in mice by modulating the gut microbiota. Scientific reports. 2016 ; 6(1), 32953.
24.Baker, J. L., Shriner, D., Bentley, A. R., & Rotimi, C. N. Pharmacogenomic implications of the evolutionary history of infectious diseases in Africa. The pharmacogenomics journal. 2017 ; 17(2), 112-120.
25.Lu, Y., Yuan, X., Wang, M., He, Z., Li, H., Wang, J., & Li, Q. Gut microbiota influence immunotherapy responses: mechanisms and therapeutic strategies. Journal of hematology & oncology. 2022 ; 15(1), 47.
26.Stancu A. L. Gut Microbiome and the Response to Immunotherapy in Cancer. Discoveries (Craiova, Romania). 2018 ; 6(3), e84.
27.Davidovics, Z. H., Michail, S., Nicholson, M. R., Kociolek, L. K., Pai, N., Hansen, R., Schwerd, T., Maspons, A., Shamir, R., Szajewska, H., Thapar, N., de Meij, T., Mosca, A., Vandenplas, Y., Kahn, S. A., Kellermayer, R., & FMT Special Interest Group of the North American Society of Pediatric Gastroenterology Hepatology, Nutrition, the European Society for Pediatric Gastroenterology Hepatology, Nutrition Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection and Other Conditions in Children: A Joint Position Paper From the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. Journal of pediatric gastroenterology and nutrition. 2019 ; 68(1), 130–143.
28.Cristofori, F., Dargenio, V. N., Dargenio, C., Miniello, V. L., Barone, M., & Francavilla, R. Anti-Inflammatory and Immunomodulatory Effects of Probiotics in Gut Inflammation: A Door to the Body. Frontiers in immunology. 2021 ; 12, 578386.
29.El-Sayed, A., Aleya, L., & Kamel, M. The link among microbiota, epigenetics, and disease development. Environmental Science and Pollution Research. 2021 ; 28, 28926-28964.
30.Blanton, L. V., Barratt, M. J., Charbonneau, M. R., Ahmed, T., & Gordon, J. I. Childhood undernutrition, the gut microbiota, and microbiota-directed therapeutics. Science. 2016 ; 352(6293), 1533-1533.
31.Rajakovich, L. J., & Balskus, E. P. Metabolic functions of the human gut microbiota: the role of metalloenzymes. Natural product reports. 2019 ; 36(4), 593-625.
32.Wang, Z., Roberts, A. B., Buffa, J. A., Levison, B. S., Zhu, W., Org, E., ... & Hazen, S. L. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. Cell. 2015 ; 163(7), 1585-1595.
33.Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D., Hirschfield, G. M., Hold, G., ... & Hart, A. The gut microbiota and host health: a new clinical frontier. Gut. 2016 ; 65(2), 330-339.
34.Singh, R. K., Chang, H. W., Yan, D., Lee, K. M., Ucmak, D., Wong, K., Abrouk, M., Farahnik, B., Nakamura, M., Zhu, T. H., Bhutani, T., & Liao, W. Influence of diet on the gut microbiome and implications for human health. Journal of translational medicine. 2017 ; 15(1), 73.
35.Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., ... & Segal, E. Personalized nutrition by prediction of glycemic responses. Cell. 2015; 163(5), 1079-1094.

http://pmjournal.ir Original Article

Spring 2023, Volume 8, Issue 29 (14-24)

Exploring the Attitudes, Beliefs and Perceptions of Undergraduate and Graduate Students in Bangladesh towards Precision Medicine and Pharmacogenomics Practice: A Qualitative Study

Nashrifa Israt Khanom[1,2], Saima Nur[1,3], Sheikh Sabrina Mustari[1,4], Omme Jamila[1,5], Abu Sa A Dat[1,6], Md Mehade Hasan[1,6], Mushfiquzzaman Mahim[1,7], Farhana Faiza Bristy[1,8], Nasrin Tamanna[1,9,11], Md.Moshrraf Hossen[1,10,11], Swapan Kumar Das[1,12], Tanvir Ahmed[1,13], Md. Ashrafuzzaman Sapon[11], Md. Shafiqul Islam[1,14], Md. Monirul Islam[1*]

[1]Division of Pharmacogenomics & Precision Medicine Research, BioIcon Academy, Dhaka, Bangladesh.
[2]Depertment of Theoretical & Computational Chemistry, University of Dhaka, Dhaka, Bangladesh.
[3]Department of Biotechnology and Genetic Engineering, University of Development Alternative Dhaka, Bangladesh.
[4]Department of Genetic Engineering and Biotechnology, East West University, Dhaka, Bangladesh.
[5]Department of Biochemistry and Molecular Biology, Tejgaon College, National University, Dhaka, Bangladesh.
[6]Department of Biochemistry and Molecular Biology, Siddheswari College, Dhaka, Bangladesh.
[7]Science Group, Dhaka Residential Model College, Dhaka, Bangladesh.
[8]Department of Pharmaceutical Sciences, North South University, Dhaka, Bangladesh.
[9]Department of Applied Genomics, Kongju National University, Chungcheongnam-do, South Korea.
[10]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh.
[11]Department of Genetic Engineering and Biotechnology, Islamic University, Kushtia, Bangladesh.
[12]Production Department, Eskayef Bangladesh Limited, Dhaka, Bangladesh.
[13]Department of Emergency & Outdoor, Islami Bank Central Hospital, Kakrail, Dhaka, Bangladesh.
[14]Department of Physical Science, Independent University Bangladesh, Dhaka, Bangladesh.

**Corresponding author: Md. Monirul Islam, Division of Pharmacogenomics & Precision Medicine Research, BioIcon Academy, Dhaka, Bangladesh.Email: info@thebioicon.com.*

DOI: 10.22034/pmj.2023.705459

Submitted: 2023-02-27
Accepted: 2023-05-10

Keywords:
Pharmacogenomics
Precision Medicine
Health
Therapeutic strategies
Genetic testig
Social implication

Abstract:
The aim of this study is to investigate the attitude, beliefs, and perceptions among undergraduate and graduate students toward precession medicine (PM) and pharmacogenomics (PGx) practice. A cross-sectional survey is conducted amongst students from different universities in Bangladesh.The results of the survey showed that the majority of students had a positive attitude towards precision medicine and pharmacogenomics, perceiving it as a means to improve diagnosis and treatment accuracy. Furthermore, many students also expressed a willingness to learn more about precision medicine and pharmacogenomics, suggesting that there is potential for these practices to be utilized in Bangladesh. Particularly in this study, 337 students from life science and relevant programs participated. From this study, it is shown that 84% of graduate students and 74% of undergraduate students thought PM is a promising healthcare model. In addition, 39% of students are highly encouraged to pursue their post-graduation in the subject areas of PGx and PM to support patients. The majority (62%) thought that patient privacy was the ethical concern most closely related to pharmacogenomic testing, while 19% of respondents thought that data confidentiality was the key issue. The results provide insight into the potential of precision medicine and pharmacogenomics in Bangladesh and suggest that further research into the attitudes of healthcare professionals should be conducted in order to take full advantage of the potential of these practices.

INTRODUCTION

Precision medicine is a novel approach to medical care that considers a person's genetic background, lifestyle, and environmental circumstances. It has gained popularity in recent years (1, 2). It is a strategy that is made significant by molecular diagnostics and contradicts the conventional method of treating all patients in the same state with the same medication and

dosage (3). However, personalized medicine uses data about a person's particular genes or proteins to treat disease (4). it has the ability to shape various aspects of clinical practice and enhance early diagnosis to the treatment of disease (5). The study of numerous genes or gene patterns and simultaneous examination of their structure and expression are required for pharmacogenomics practice (6). It is also important to investigate how variations in the human genome affect an individual's response to particular drugs. In twenty centuries, the human genome project (HGP) reported that humans have approximately 20,500 genes and that 99.5 percent of the genes are analogous, only 0.5 percent of the genes have differences that are accountable for the specific groups and cause-specific diseases (7, 8). Therefore, the emphasis has shifted to using genetic techniques to identify markers for therapeutic response.

The number of SNPs linked to medication reactions will increase at a never-before-seen rate during the coming years. The task is to sort through the pertinent SNPs and show the clinical validity and efficacy of these SNPs as Pharmacogenomics indicators (9). In the human genome NSP is the most prevalent type of DNA sequence variation (10). Approximately 11 million SNPs in the human genome which an average one SNPs found in 1300 base pairs. It is act as a biological markers and determine an individual's response to certain drugs and risk of developing diseases (11, 12).

Adverse drug reactions (ADRs) are the fourth major cause of mortality in the United States, and it is thought that 2.74 million ADRs and 128,000 fatalities are caused each year by prescription medications(13). As a result, one out of every five wounds or deaths among hospitalized patients are caused by ADRs, which have an annual cost of $136 billion greater than the combined expenditures of treating diabetes and cardiovascular disease (13). The goal of PGx discoveries is to maximize the advantages of drugs while minimizing any negative effects and healthcare expenses (12).

According to the recent pharmacogenomics report, the Food and Drug Administration's (US-FDA) collection of medications that have been labeled before use currently includes more than 350 drugs (14). These drugs are often referring to multiple pharmacogene, resulting in ~15% of all approved drugs having pharmacogenomics information on their labels (8, 15) In order that pharmacogenomics and personalized medicine approach played a crucial role in preventing genetic disorder. However, the concern arises with genetic testing that must satisfy specific requirements with respect to their clinical utility, clinical validity, and analytical validity before use in clinical context (16, 17). In addition, concerns about the security and privacy of a patient's pharmacogenomics data are also raised by personalized medicine approaches (18).

The public's awareness of the molecular uses and characterization of PGx and PM during the COVID-19 outbreak in Bangladesh has increased because to the advancement of genome sequencing research.(19, 20, 21, 22). Surprisingly, there are presently no local studies that address the public's knowledge and perceptions about PM, PGx, and genetic testing, and there is no educational program at the undergraduate and graduate levels. Information regarding PM, PGx and its testing are very low, but few studies are available from Asia (2, 4). The majority of the report arrived from USA and Europe (23, 24). On the other hand, knowledge and awareness of these are crucial since they could be used as a guide when developing national policy and curriculum.

This research article explores the attitudes, beliefs, and perceptions of undergraduate and graduate students in Bangladesh towards precision medicine and pharmacogenomics practice. A qualitative approach was employed in order to gain insight into the participants' perspectives. The focus of this survey was to analysis insight into the views and opinions of students in Bangladesh towards precision medicine and pharmacogenomics practice, and to provide a foundation for future research on the topic. This study is significant as it provides valuable insight on the views of the student population towards precision medicine and pharmacogenomics practice, which in turn can be used to provide a better understanding of the general population's perspective, can also be used to inform future research on the topic, as well as provide a platform for further discussion on the implications of precision medicine and pharmacogenomics practice (25, 26).

METHODOLOGY

Study Design

Cross-sectional research was carried out over the course of four months, from July 1 to October 25, 2022. The purpose of this population-based cross-sectional study was to examine the understanding, attitudes, and application of pharmacogenomics and personalized medicine. A combination of qualitative and quantitative data was used to conduct the study. Current students from a number of Bangladeshi universities qualified as participants. The total number of 337 graduate and undergraduate students contributing to the survey were from life science backgrounds, as well as those with backgrounds in various fields outside of molecular life science and health science. They could communicate in English, ranged in age from 18 to 60, and were citizens of Bangladesh with various socioeconomic backgrounds and educational institutions. On the basis of a question from the Mahmutovic et al. study, an online questionnaire was made and updated and

given to participants to answer in order to learn how undergrads studying molecular life sciences and health felt about PGx and PM (3). Each and every participant was fully informed of the study's purpose prior to the data collection.

Sampling and Data collection

The 39 questions in the survey were separated into three groups as follows: Part 1 consists of demographic information, including ages, gender, and educational attainment. About 15-20 question related to pharmacogenomics and personalized medicine included in part 2 and was concerned through knowledge and awareness related questionnaire. Part-3 of the survey included five to seven multiple-choice questions about respondents' opinions about pharmacogenomics and the practice of personalized medicine. Definition of pharmacogenomics/Pharmacokinetics test were given to the respondents which were included in the introduction of the survey. In this survey these question answers where yes/no/I don't know (not sure). The survey also included multiple-choice questions and a Likert scale for rating of agreement with various statements like agree, disagree, no opinion, neutral. The aim and objectives of this study were described in the introductory cover page attached to the questionnaire and invited students to participate in it.

STATISTICAL ANALYSIS

All categorical variables were presented as frequencies and percentages, including participant professional information, demographics and response to question concerning participants' opinions of PGx and PM. Data analysis was conducted using Microsoft excel and SPSS software. In order to calculate proportions, descriptive statistics were utilized. Chi-square test were used to determine the relationship between demographic factors and response and understanding, perception, and practice of pharmacogenomics and precision medicine. The p values were determined via appropriate statistical test. All statistical tests were performed with a significance threshold of 5%, and the odd ratio (OR) and correspondence 95% confidence intervals (CI) were computed.

RESULT

Participants' demographics

Table 1 summarizes the student's demographic information as well as their employment history. The current study included 337 students who consented to fill out the questionnaire. Most of the students

Table 1. Student's demographic characteristics and professional information.

	Total	Undergraduate student	Graduate student	* MLS & HS	**Non-MLS & HS	P value
Gander						
Male	157	111	46	131	26	
Female	180	146	34	166	14	0.025
Age						
<19	7	7	0	3	4	
19-26	262	234	27	234	28	
27-40	67	16	51	60	7	P<1
41-50	2	0	2	1	1	
51-60	0	0	0	0	0	
>60	0	0	0	0	0	
Number of family member						
<4	61	40	21	50	11	
4-6	227	177	50	200	27	0.094
6-10	31	27	4	29	2	
>10	18	13	5	18	0	
Family income						
<25000 TK	89	72	17	81	8	
25000-50000 TK	147	107	40	132	15	0.546
50000-100000 TK	82	63	19	66	16	
>100000 TK	19	15	4	18	1	
Level of education						
HSC	26	26	0	23	3	
BSc	231	231	0	207	27	
MSc	77	0	77	67	10	P<1
MPhil	0	0	0	0	0	
PhD	3	0	3	3	0	

***MLS & HS**= Molecular Life Science & Health Science**,** this area includes Biochemistry & Molecular Biology, Genetics and Biotechnology, Medicine, Health Studies, Microbiology, Pharmaceutical Sciences. ****Non-MLS & HS**= Non- Molecular Life Science & Health Science**,** it includes Computer Science, Electrical and Electronic Engineering, Journalism, Anthropology, BBA

were aged between 19–26 years. Among the 337 participants, 257 were undergraduates and 80 were graduates. Among them, 180 (53%) were female and 157 (47%) were male.

Students' attitudes towards pharmacokinetics practice and personalized medicine

Various question and responses used to assess pharmacogenomics knowledge were shown in table 2.

Table 2. Students' attitudes towards pharmacogenetics practice and personalized medicine

	Total	Undergraduate student	Graduate student	*MLS & HS	Non-MLS & HS	P value
Do you know some genetic disease transmitted by inheritance from one generation to another?						
Yes	284	219	65	259	25	0.678
No	32	22	10	23	9	
Don't know	10	7	3	9	1	
Not sure	11	9	2	6	5	
Do you think; Genetic Counselor can help you to refer to the right doctor about genetic disease related issues?						
Yes	284	212	72	254	30	0.175
No	13	9	4	11	2	
Don't know	18	16	2	16	2	
Not sure	22	20	2	16	6	
Do you know; action of drugs can vary person to person. For this reason, personalized medicine is very important.						
Yes	303	230	73	268	35	0.024
No	12	6	6	12	0	
Don't know	9	8	1	9	0	
Not sure	13	13	0	8	5	
Do you know about 'companion diagnostics'?						
Yes	115	84	31	110	5	0.078
No	161	127	34	136	25	
Don't know	33	21	12	29	4	
Not sure	28	25	3	22	6	
Have you heard about personal genome testing companies?						
Yes	152	104	48	136	13	0.009
No	136	116	20	120	22	
Don't know	30	22	8	24	4	
Not sure	19	15	4	18	1	
Have you ever had an adverse drug reactions?						
Yes	106	73	33	95	11	0.155
No	167	131	36	147	20	
Don't know	44	36	8	38	6	
I have never taken any medication	20	17	3	17	3	
Have you ever found that a particular drug not work for you?						
Yes	137	103	34	126	11	0.697
No	123	92	31	113	10	
Don't know	56	44	12	42	14	
I have never taken any medication	21	18	3	16	5	
To what extend do you think that genes influence your health?						
Completely	127	81	46	120	7	0.0001
Moderately	129	110	19	113	16	
Not at all	19	18	1	14	5	
Don't know	62	48	14	50	12	
Would you consider having a genetic test done to find out what illness you might develop in the future?						
Yes	248	183	65	224	24	0.080
No	43	33	10	35	8	
Don't know	46	41	5	38	8	
Do you agree that personalized medicine represents a new and promising healthcare model?						
Yes	262	195	67	239	23	0.334
No	17	14	3	12	5	
Don't know	58	48	10	46	12	
Would you consider contacting a personal genome testing company and ordering a pharmacogenomic test for yourslef?						
Yes	157	106	51	150	7	0.0002
No	68	50	18	56	12	
Not sure	77	70	7	62	15	
Don't know	35	31	4	29	6	

Continue the table 2. Students' attitudes towards pharmacogenetics practice and personalized medicine

If a pharmacogenomics test revealed that a prescribed drug would either be ineffective or cause severe side effect, would you take the drug anyway?						
Take the drug anyway	19	16	3	14	5	0.108
Accept the test result, and not take the drug	143	100	43	133	10	
Accept the test result and take the drug only if the disease might be life-threatening	127	104	23	110	17	
Not sure	48	37	11	40	8	
To what extent do you think that genes influence your health?						
Completely	134	87	47	126	8	0.001
Moderately	123	104	19	106	17	
Not at all	18	14	4	14	4	
Don't know	62	52	10	51	11	
If you know your genetic tendency to develop a disease, would you be ready to make necessary changes in your lifestyle, to reduce disease risk?						
Yes	289	217	72	259	30	0.463
No	13	12	1	9	4	
Not sure	18	15	3	15	3	
Don't know	17	13	4	14	3	
Do you agree that personalized medicine represents a new and promising healthcare model?						
Yes	263	195	68	240	22	0.179
No	18	14	4	12	6	
Don't know	56	48	8	44	12	

*MLS & HS= Molecular Life Science & Health Science

Replies to all questions regarding their awareness and attitude related to genetic testing, pharmacogenomics, and personalized medicine are also shown here. Among the participants from the field of medicine, pharmacy, health science, genetics, and bioengineering roughly 40% did not work for a particular drug, while 31% of these students had an unfavorable medicine response.

Significance of pharmacogenomics education

The findings in Table 3 and Table 4 show that medical, pharmacy and heath studies students have similar perspectives on their education program and upcoming diplomacies for PGx. Overall, 84% of graduates and 76% of undergraduates believed that PM is promising healthcare model. The majority of undergraduates, 82% (212/257) agreed that PGx should be relevant to their curriculum, and 42% (108/257) thought their program were well organized for PGx. The curriculum wasn't well-designed for PGx, according to 31% of respondents (81/257) and 39% (100/257) want to running their next learning degree (masters, PhD, specializations) in the area of personalized medicine. According to our findings, students' opinions toward their course of study and their desire to pursue postgraduate research in the field of personalized medicine are both highly influenced by the subject of study. When compare to other responders, it seems that more Biochemistry and Molecular biology students would like to pursue postgraduate study in this area. Additionally, our findings imply that students are more likely to pursue postgraduate studies related the field of personalized medicine if the program is well designed to provide them a sufficient understanding of PG.

In their future practices, more than 70% of undergraduates and recent graduates feel that they are able to identify patients who might get advantage from genomic identification, in addition they can address patients' inquiries about PG and PM and recognize medications that call for pharmacogenomics testing before being administered to the patient.

Students' awareness about the ethical, legal and social implications

In this survey study it is seen that 54% of the students are conscious about ethical issues related to genetic testing and 60% of those are believe that privacy of the patients is heights' concerning issue associated with pharmacokinetic testing, whereas just 19% thought that the main issue is the confidentiality of data protection. The racial issue, non-incidental findings, and stigma possess 5%, 7%, and 5% of the other ethical issue. Our findings indicate that 74% of students seem that disclosing of PGx test results might be an unlawful practice. This concern was echoed by students in all faculties. Furthermore, 53% of students trust that revealing an unfavorable test result would be a disadvantage at the workplace or in job-searching and are also worried that they would feel "helpless" or "pessimistic".

DISCUSSION

One of our study's unique features is that it is the

Table 3. Students opinion regarding the study curriculum and their future plans in pharmacogenomics

	Total	Undergraduate student	Graduate student	*MLS & HS	Non-MLS & HS	P value
Pharmacogenomics should be an important part of my study curriculum.						
Agree	283	212	71	264	19	0.029
Disagree	1	1	0	0	1	
Neutral	39	36	3	24	15	
No opinion	14	8	6	9	5	
Do you think that the curriculum of your study program is well designed for understanding pharmacogenomics?						
Yes	158	108	50	148	10	0.010
No	100	81	19	82	18	
Don't know	36	32	4	29	7	
Not sure	43	36	7	38	5	
Would you like to continue your postgraduate education (Masters, PhD, specialization) in the field of personalized medicine?						
Yes	150	100	50	148	2	0.001
sure	102	83	19	89	13	
Don't know	40	36	4	30	10	
No	45	38	7	30	15	

*MLS & HS= Molecular Life Science & Health Science

Table4. Students attitudes towards continued education in pharmacogenomics

	Total	Undergraduate student	Graduate student	*MLS & HS	Non-MLS & HS	P value
In my future practice, I should be able to identify pationts that could benefit from genetic testing.						
Agree	251	191	60	231	20	0.0002
Disagree	5	0	5	5	0	
Neutral	41	36	5	29	12	
No opinion	40	30	10	32	8	
In my future practice, I should be able to answer patient's questions regarding pharmacogenomics and personalized medicine.						
Agree	250	189	61	228	22	0.862
Disagree	6	5	1	3	3	
Neutral	46	37	9	40	6	
No opinion	35	26	9	26	9	
In my future practice, I should be able to identify drugs that would require pharmacogenomics testing prior to their administration to the patient.						
Agree	228	170	58	212	16	0.559
Disagree	16	12	4	14	2	
Neutral	48	37	11	38	10	
No opinion	45	38	7	33	12	

*MLS & HS= Molecular Life Science & Health Science

first study to examine graduate and undergraduate students from multiple different universities in Bangladesh about their knowledge of and attitudes on the part of pharmacogenomics and precision medicine. Our findings indicated that participants from life science and health science are typically conscious for pharmacogenomics test & have a basic understanding of personal genome testing companies. Students in non-molecular life sciences, as opposed to, are fewer conscious for this medicine and not concerned in using PM may a good healthcare model than students in molecular life science. Here, we also established that most of the graduate and undergraduate students think that PGx should play a significant role in their academic program and higher than 50% of these students would like to acquire there next study program related to the field of personalized medicine (27, 28). Most of the faculties may not have PGx-related courses included in their curriculum, as just one-third of all students who took part in our survey believed that their study curriculum is properly prepared to understand PGx.

In a recent survey, it was discovered that the vast majority of the students in California's eight pharmacy schools were knowledgeable about pharmacogenomics, concurred that pharmacogenomics is significant for future pharmacists, and expressed interest in following a PGx residency, fellowship, or career. However, Latif(29) noted that only a basic understanding of PGx was being taught in the USA by 2005, emphasizing the requirement to include PGx in the pharmacy curriculum.

In recent year Direct-to-consumer Genetic Testing (DTCGT) companies have risen, offering substitute information on genetic testing (GT) and personalized

Table 5. Students awareness and opinion regarding the ethical, legal, and social implication

	Total	Undergraduate student	Graduate student	*MLS & HS	Non-MLS & HS	P value
Are you aware of different ethical aspects of genetic testing?						
Yes	182	135	47	167	15	0.015
No	84	69	15	70	14	
Not sure	36	32	4	29	7	
Don't know	35	21	14	31	4	
What ethical isues do you believe might be related to genetic or pharmacogenomics testing?						
Patient privacy	210	147	63	186	24	0.001
Racial issues	16	16	0	14	2	
Non-incidental findings	23	21	2	22	1	
Data confidentiality	64	49	15	56	8	
Stigma	18	18	0	15	3	
Other	6	6	0	4	2	
Are you worried about the possibility that the result of a pharmacogenomics test may be passed to unauthorized persons?						
Very worried	135	86	49	124	11	0.0001
Not worried	29	25	4	26	3	
Slightly worried	113	98	15	99	14	
I don't know	60	48	12	48	12	
In case of any unfavorable test result should be disclosed, do you believe that you would be disadvantages at work or job-seeking?						
Yes	177	123	54	161	16	0.007
No	51	41	10	44	7	
No opinion	109	93	16	92	17	
In case of an unfavorable test results, do you believe that you would feel "helpless" or "pessimistic"?						
Yes	178	126	52	158	20	0.003
No	73	54	19	65	8	
No opinion	86	77	9	74	12	
In case of an unfavorable test result, do you believe that you would feel "different" or "inadequate"?						
Yes	182	129	53	164	18	0.014
No	64	49	15	56	8	
No opinion	91	79	12	77	14	

*MLS & HS= Molecular Life Science & Health Science

medicine (PM), while highlighting the remarkable benefits of genomic medicine for specific healthcare management. Although students' knowledge about genetic testing, precision medicine, and pharmacogenomics may be largely based on information and advertisements from the direct-to-consumer genetic testing (DTCGT) industry, which may contain inaccuracies and overstatements, rather than more accurate information acquired from their academic curriculum. (30, 31). As seen, few numbers of students expecting to continue their doctoral lessons in PM, undergraduates find it challenging to acquire a great interest in future consideration of such subjects without a thorough knowledge of PM, PGx, and GT. So, by focusing more attention and resources on academic study and profession development in PM and PGx, there is a high chance that genomic medicine will be promoted thanks to a strong base of knowledge and widespread support.

According to our finding, 76% of undergraduate students believed PM is an encouraging healthcare model, and 54% said they would think about getting a genetic test. Initial instruction in genetics and genomics starts in high school in Bangladesh, but it does so in kindergarten through primary school in other western nations like the United States. Kindergarten students in the USA are exposed to the fundamental ideas of genetic inheritance through the application of relatable cases, such as cats giving birth to kittens with distinct markings, to show how features can vary. Due to this, the educational system in the USA provided evidence that genomic education could be implemented and a solid foundation in genetics could be built at an early learning stage (32, 33). In recent years' inadequate education and talent progress in PM and PGx practice may be exaggerated due to slow local progress of its practice. The practice of PGx in Bangladesh is still in primary level compared to other countries.

Negative attitude toward genetic testing results due to ethical, legal and social implications

It has been established that students who took

Table 6. Students awareness toward diagnosis of diseases and treatment option

	Total	Undergraduate student	Graduate student	*MLS & HS	Non-MLS & HS	P value
Have you been diagnosed with any of the following diseases? You can choose multiple options.						
Cardiovascular (heart problems, atherosclerosis, hypertension)	19	16	3	17	2	0.700
Psychiatry (depression, anxiety)	48	38	10	41	7	
Oncology (any type of cancer)	4	3	1	4	0	
Metabolic diseases (diabetes, metabolic syndrome)	23	20	3	22	1	
No	232	172	60	203	29	
Other	11	8	3	10	1	
Did you ever take a drug that is used to treat any of the following diseases? You can choose multiple options.						
Cardiovascular	14	13	1	14	0	0.160
Psychiatry	16	14	2	12	4	
Metabolic disease (Diabetes)	13	11	2	12	1	
Oncology	2	1	1	2	0	
I do not take drugs	266	195	71	235	31	
Other	26	23	3	22	4	
How much money are you willing to spend to examine the effectiveness of a specific drug in your body using a pharmacogenomic test?						
<5000 TK	160	110	50	145	15	0.027
5000-8000 TK	33	25	8	27	6	
8000-12000 TK	11	9	2	11	0	
>12000 TK	10	8	2	10	0	
Not sure	123	105	18	104	19	
Do you think, cost of Precision Medicine & Pharmacogenomics testing will be reduced in the near future like general diagnostics screening?						
Yes	176	137	39	155	21	0.018
No	41	24	17	41	0	
Don't know	65	49	16	56	9	
Not sure	55	47	8	45	10	
Do you believe that in the future pressure may be exerted on patients to agree to perform a pharmacogenomics test?						
Yes	201	141	60	182	19	0.004
No	51	42	9	43	8	
No opinion	85	74	11	72	13	

*MLS & HS= Molecular Life Science & Health Science

part in our survey are aware of the various ethical issues surrounding genetic issue and its testing. However, from our survey it is showed that most of the students seems to be concerned about parents' secrecy and data privacy. More than 40% of Bangladeshi undergraduates demonstrated a negative outlook in the event of a poor GT result, including feelings of "helplessness or pessimism," "different or inadequate," and "disadvantaged" job seeking, with students older than the age of 19 more inclined to agree with this statement(34).

Typically, Asians are more pessimistic than other ethnic groups, indicates that the propensity of pessimism among local undergraduates are more pessimistic. According to a research by Chang et al., Asians Americans are generally extra doubtful than Caucasian Americans (35).Similarly, results were found in another study by Lee et al., which showed that Caucasia American students and Chinese American students both had higher levels of pessimism than mainland Chinese students and Chinese American students, respectively (36). Although the tendency of pessimism about poor GT results is particularly pronounced and widespread among Bangladeshi undergraduates, the highlighting causes of pessimism and potential solutions to reverse the trend should be thought and carefully addressed. Surprisingly, our study showed that nearly half of all respondents were concerned that PGx test results would be disclosed to unauthorized parties. Students who are concerned that PGx testing show further hazard issues for

former illness would equally feel "different" and "inadequate" in the event of negative test results. Otherwise, numerous participants claimed that they wouldn't feel "helpless," "pessimistic," "different," or "inadequate." This means that every person would respond to the genetic test results differently.

Patients are thought to need sufficient counseling in order to understand the significance of the test results in relation to their particular health.(37, 38). One of the most significant findings of our study is for the national health service it is necessary to recruit a diverse group of students in three different settings like medicine, pharmacy, and health studies. Students from genetics and other non-molecular life science and non-health science departments are also important. Our findings were further strengthened by the comparison of the thoughts and attitudes of students who had taken the PGx course and those who had not. Our survey accelerates students' interest for learning more about PGx.

CONCLUSION

This study provides evidence of how undergraduate and graduate students in Bangladesh perceive PM and PGx. Our findings show that, with the exception of graduate students from Bangladesh, the majority of undergraduate students who participated in our survey are enrolled in life science programs. However, they believe PM is a promising healthcare model but their knowledge, understanding, technologies used for testing, its applications are very poor. However, the majority of students who are studying molecular life and health science want to learn more about this program. This fact suggests that study programs in this field should be developed in order to ensure better service regarding personalized medicine. Therefore, it is necessary to improve coordination between universities, healthcare organizations, and governing bodies in order to include more training and continuing education themes about pharmacogenomics and personalized medicine. In order to ensure the widespread clinical adoption of personalized medicine, it is crucial to expand the pharmacogenomic path of biological education. Also, this study is significant as it provides valuable insight into the views of precision medicine and pharmacogenomics practice, which can be used better understand the general population's perspective, and can also be used to inform future research on the topic.

Acknowledgements

The authors are thankful to the study participants. Also, the authors are grateful to the faculty members of different Universities to encourage their students to participate this research in Bangladesh.

Author Contributions

The main conceptual ideas, technical details and questioner are made by Md Monirul Islam. All authors are participating in data collection, analysis, reading and processing of the manuscript. All authors read, edition and approved for the publication.

Conflict of Interest

The authors announce that there is no conflict of interest for disclosing and publication of this paper.

REFERENCE

1.Prajapat, M., Shekhar, N., Sarma, P., Avti, P., Singh, S., Kaur, H., Bhattacharyya, A., Kumar, S., Sharma, S., Prakash, A., & Medhi, B. Virtual screening and molecular dynamics study of approved drugs as inhibitors of spike protein S1 domain and ACE2 interaction in SARS-CoV-2. Journal of Molecular Graphics and Modelling. 2020;101, 107716.

2.Cheung, N. Y. C., Fung, J. L. F., Ng, Y. N. C., Wong, W. H. S., Chung, C. C. Y., Mak, C. C. Y., & Chung, B. H. Y. Perception of personalized medicine, pharmacogenomics, and genetic testing among undergraduates in Hong Kong. Human Genomics. 2021 ; 15(1), 1–12.

3.Mahmutovic, L., Akcesme, B., Durakovic, C., Akcesme, F. B., Maric, A., Adilovic, M., Hamad, N., Wjst, M., Feeney, O., & Semiz, S. Perceptions of students in health and molecular life sciences regarding pharmacogenomics and personalized medicine. Human Genomics. 2018; 12(1), 1–15.

4.Saud, K., & Syed, W. Knowledge, Attitude and Perception of University of Ibadan Pharmacy Students on Veterinary Pharmacy Practice. Nigerian Journal of Pharmacy. 2022 ; 56(1).

5.Moses III, H., & Martin, J. B. Academic Relationships With Industry. Jama.2001 ; 285(7), 933.

6.Dhawan, D., & Padh, H. Pharmacogenomics and personalized medicine for cancer. In Omics for Personalized Medicine. 2013.

7.Abou Diwan, E., Zeitoun, R. I., Abou Haidar, L., Cascorbi, I., & Khoueiry Zgheib, N. Implementation and obstacles of pharmacogenetics in clinical practice: An international survey. British Journal of Clinical Pharmacology. 2019 ; 85(9), 2076–2088.

8.Relling, M. V., & Evans, W. E. Pharmacogenomics in the clinic. Nature.2015 ; 526(7573), 343–350.

9.Norton, R. Pharmacogenomics: The Search for Individualized Therapies. In Drug Discovery Today. 2002 ; (Vol. 7, Issue 21).

10.Robert, F., & Pelletier, J. Exploring the Impact of Single-Nucleotide Polymorphisms on Translation. Frontiers in Genetics. 2018 ; 9(October), 1–11.

11.Madsen, B. E., Villesen, P., & Wiuf, C. A periodic pattern of SNPs in the human genome. Genome Research. 2007 ; 17(10), 1414–1419.

12.Reisberg, S., Krebs, K., Lepamets, M., Kals, M., Mägi, R., Metsalu, K., Lauschke, V. M., Vilo, J., & Milani, L. Translating genotype data of 44,000 biobank participants into clinical pharmacogenetic recommendations: challenges and solutions. Genetics in Medicine. 2019 ; 21(6), 1345–1354.
13.Shepherd, G., Mohorn, P., Yacoub, K., & May, D. W. Décès suite aux effets indésirables rapportés aux États Unis en utilisant les statistiques de l'état civil entre 1999-2006. Annals of Pharmacotherapy. 2012 ; 46(2), 169–175.
14.Koutsilieri, S., Tzioufa, F., Sismanoglou, D. C., & Patrinos, G. P. (2020). Unveiling the guidance heterogeneity for genome-informed drug treatment interventions among regulatory bodies and research consortia. Pharmacological Research. 2020 ; 153, 104590.
15.Kinsella, M., & Monk, C. NIH Public Access. 2012 ; 23(1), 1–7.
16.Burke, W. Clinical validity and clinical utility of genetic tests. Current Protocols in Human Genetics, SUPPL. 2009 ; 60, 1–7.
17.Issa, A. M., & Keyserlingk, E. W. Apolipoprotein E genotyping for pharmacogenetic purposes in Alzheimer's disease: Emerging ethical issues. Canadian Journal of Psychiatry. 2000 ; 45(10), 917–922.
18.Robertson, J. A. Consent and privacy in pharmacogenetic testing. Nature Genetics. 2001 ; 28(3).
19.Khan, S., Akter, S., Goswami, B., Habib, A., Banu, T. A., Barton, C., Osman, E., Samir, S., Arjuman, F., Hasan, S., & Hossain, M. Whole genome mapping and identification of single nucleotide polymorphisms of four Bangladeshi individuals and their functional significance. BMC Research Notes. 2021 ; 14(1), 1–6.
20.Ahammad, I., Hossain, M. U., Rahman, A., Chowdhury, Z. M., Bhattacharjee, A., Das, K. C., Keya, C. A., & Salimullah, M. Wave-wise comparative genomic study for revealing the complete scenario and dynamic nature of COVID-19 pandemic in Bangladesh. PLoS ONE. 2021 ; 16(9 September), 1–21.
21.Akter, S. (n.d.). Genome sequencing and genetic characterization of 17 SARS-CoV-2 viruses and spike protein analyses of isolates from Bangladeshi patients. Research Square. 2021; 1–20.
22.Rahman, M. M., Kader, S. B., & Rizvi, S. M. S. Molecular characterization of SARS-CoV-2 from Bangladesh: implications in genetic diversity, possible origin of the virus, and functional significance of the mutations, Heliyon. 2021; 7(8), e07866.
23.Swen, J. J., Nijenhuis, M., van Rhenen, M., de Boer-Veger, N. J., Buunk, A. M., Houwink, E. J. F., Mulder, H., Rongen, G. A., van Schaik, R. H. N., van der Weide, J., Wilffert, B., Deneer, V. H. M., & Guchelaar, H. J. Pharmacogenetic Information in Clinical Guidelines: The European Perspective. Clinical Pharmacology and Therapeutics. 2018; 103(5), 795–801.
24.Volpi, S., Bult, C. J., Chisholm, R. L., Deverka, P. A., Ginsburg, G. S., Jacob, H. J., Kasapi, M., McLeod, H. L., Roden, D. M., Williams, M. S., Green, E. D., Rodriguez, L. L., Aronson, S., Cavallari, L. H., Denny, J. C., Dressler, L. G., Johnson, J. A., Klein, T. E., Leeder, J. S., Relling, M. V. Research Directions in the Clinical Implementation of Pharmacogenomics: An Overview of US Programs and Projects. Clinical Pharmacology and Therapeutics. 2018; 103(5), 778–786.
25.Bienfait, k., Aparna Chhibber, Jean-Claude Marshall, Martin Armstrong, Charles_Cox, Peter M.Shaw & Charles Paulding. Current challenges and opportunities for pharmacogenomics: perspective of the Industry Pharmacogenomics Working Group (I-PWG). HumanGenetics. 2022;141. P1165–1173.
26.Cecchin, E., and Gabriele Stocco. Pharmacogenomics and Personalized Medicine. Genes.2020; 11(6), 679.
27.Guy, J. W., Patel, I., & Oestreich, J. H. Clinical Application and Educational Training for Pharmacogenomics. Pharmacy. 2020; 8(3), 163.
28.Nickola, T. J., Green, J. S., Harralson, A. F., & Obrien, T. J. The current and future state of pharmacogenomics medical education in the USA. Pharmacogenomics. 2012; 13(12), 1419–1425.
29.Latif, D. A., & McKay, A. B. Pharmacogenetics and pharmacogenomics instruction in colleges and schools of pharmacy in the United States. American Journal of Pharmaceutical Education. 2005; 69(2), 152–156.
30.Zayts, O., & Luo, Z. Commodification and marketisation of genetic testing through online direct-to-consumer platforms in Hong Kong. Discourse and Communication. 2017; 11(6), 630–647.
31.Rafiq, M., Ianuale, C., Ricciardi, W., & Boccia, S. Direct-to-consumer genetic testing: A systematic review of european guidelines, recommendations, and position statements. Genetic Testing and Molecular Biomarkers. 2015; 19(10), 535–547.
32.Campion, M. A., Goldgar, C., Hopkin, R. J., Prows, C. A., & Dasgupta, S. Genomic education for the next generation of health-care providers. Genetics in Medicine. 2019; 21(11), 2422–2430.
33.Learning, K., & Curriculum, A. Science Education. Physics Today. 1964; 17(11), 94.
34.Hunt, S., Wisocki, P., & Yanko, J. Worry and use of coping strategies among older and younger adults. Journal of Anxiety Disorders. 2003; 17(5), 547–560.
35.Chang, E. C. a Test of a General Negativity Hypothesis. 1996; 21(5), 819–822.
36.Brookfield, S. from the SAGE Social Science Collections . All Rights. Hispanic Journal of Behavioral Sciences. 1984; 9(2), 183–205.
37.Winkler, E. C., & Wiemann, S. Findings made in gene panel to whole genome sequencing: data, knowledge, ethics–and consequences? Expert Review of Molecular Diagnostics. 2016; 16(12), 1259–1270.

38.Howard, H. C., & Borry, P. Survey of European clinical geneticists on awareness, experiences and attitudes towards direct-to-consumer genetic testing. Genome Medicine.2013; 5(5), 0–31.

http://pmjournal.ir Original Article

Spring 2023, Volume 8, Issue 29 (25-30)

Personalized Medicine for Antibiotics: Pharmacological Displacement of Thiocolchicosidum as Antimicrobial Agent

Farhad Jafari-Berenjestanaki[1*], Faeze Hasani[2]

[1] Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Tehran Pishva Branch, Varamin, Iran.

[2] Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, North Tehran Branch, Tehran, Iran.

Corresponding author: Farhad Jafari-Berenjestanak, Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Tehran Pishva Branch, Varamin, Iran. Email: biopromeda@gmail.com

DOI: 10.22034/pmj.2023.705460

Submitted: 2023-02-18
Accepted: 2023-05-23

Keywords:
Bacterial Resistance
Pharmacology
Thiocolchicosidum

Abstract :
Due to the increasing level of bacterial antibiotic resistance (AB), it is now required to modify the dosage for customized medication using therapeutic drug monitoring. The creation of a novel treatment for clinical use, such as situations of bacterial resistance, has been hailed as a feasible, affordable, and quick alternative by the pharmaceutical industry. Therefore, the current research sought to examine the myorelaxant Thiocolchicosidum's antibacterial activity against bacterial strains. Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 25933, and Pseudomonas aeruginosa ATCC 27853 were used as the bacteria in an in vitro experimental study, along with the protocols for antibacterial activity screening, minimum inhibitory concentration (MIC), and characterization of antibacterial activity. Thiocolchicosidum, at levels varying from 0.48 to 1000 µg/mL, was the chemical. The only bacterial strains that showed any sensitivity to the myorelaxant were E. coli and P. aeruginosa, both of which had MICs of 500 µg/mL and 1000 µg/mL, respectively. Thiocolchicosidum demonstrated a bacteriostatic effect in the antimicrobial characterization test. Therefore, despite the fact that this medication is already considered safe for human use, no discernible antibacterial effects were shown in common bacterial strains. Therefore, research is required to determine how it differs from other microbes, such as various kinds of bacteria, fungus, and protozoa, in order to rule it out as a potential antibiotic material for use in industry.

INTRODUCTION

A new era of advancement in the management of bacterial diseases in individuals, agriculture, and animals was ushered in with the development of antibiotics (1). However, the emergence of multi-drug-resistant bacteria (MDR), which have considerably grown in recent years owing to antibiotics' improper handling and have become a worldwide public health concern (2), has made the use of antibiotics problematic. Since more than 70% of microorganisms are tolerant to all or part of the known antibiotics, either new antibiotic types must be developed or very toxic "last-line" antimicrobial medicines must be used in antimicrobial therapy to treat bacteria effectively, especially in critically sick patients (3). According to the research, without the development of novel compounds, antimicrobial medication resistance illnesses are predicted to cause 10 million deaths globally annually by 2050 and cost near USD 100 trillion (4).

The World Health Organization (WHO) has created a variety of measures, including regulations on the marketing, administration, and dose of antibiotics (5,6), to prevent this rising incidence of MDR illnesses. Treatment failures are produced as a result of the fact that the majority of dosages are presently administered consistently to patients without consideration for the severity of the illness or the clinical picture (7,8). This might result in sub-therapeutic or toxic doses. The use of therapeutic drug monitoring (TDM), which tracks pharmacokinetic (PK) changes to quantify medications with a narrow therapeutic index (TI) but substantial toxicity, is one of the answers.

Dual or mass-coupled chromatographic procedures using a range of detectors, such as UV or fluorescence detectors, as well as immunoassays are examples of monitoring approaches (10,11). The US Food and Drug Administration (FDA) has given its approval to several of these methods (12). However, these are costly procedures that need for specialized labs and

qualified staff.

The desire for more potent pharmacological options for clinical use and the awareness of certain medications' pleiotropic effects lead to the repositioning of pharmaceuticals, which involves using an already-known and sold drug in an unfamiliar clinical setting. The cheaper cost and previous understanding of toxicological characteristics in the body of a person are the two benefits of this technique that may be highlighted in this situation (13). Inhibitors of HMG-CoA reductase and their possible uses for neurological protection and sepsis therapy should be brought up to clarify this situation (14).

A concerning problem for world health is the rise of bacterial antibiotic resistance. The primary root of this issue is determined to be the exaggerated and ineffective administration of antibiotic medication. Given the above, there is a need for fresh antibacterial pharmaceutical treatments, and medication repurposing seems to be a workable, quick, and affordable technique in comparison to traditional drug development. The fact that this gadget received greater attention during the COVID-19 epidemic is also lauded, however, the first publication on the subject was written by researchers, and the number of articles continued to rise (15).

Therefore, the necessity for more investigations from the standpoint of medication reuse is warranted given the urgency of finding novel antibacterial medicines, the rising detection of drug-resistant strains, and the rise in complications from infectious illnesses. Due to the prospect of a novel antimicrobial agent, this research intends to evaluate how the myorelaxant Thiocolchicosidum interacts with bacteria.

METHODS

Place of the search

The experiments were conducted at the Parsian BioProducts Company (PBP) of Iran's microbiology facility.

Chemicals utilized

An injectable Thiocolchicosidum mixture (Sanofi Aventis®) with a concentration of 2 mg/ml was utilized as the study's subject in order to conduct the tests. Additionally, utilized were sterilized purified water, gentamicin as a diluent, and a control group.

Microorganisms

Four typical ATCC strains of bacteria, including *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 25933, and Pseudomonas aeruginosa ATCC 27853*, had their sensitivity assessed. Mueller-Hinton Agar (MHA), Mueller-Hinton Broth (MHB), and Brain Heart Infusion Broth (BHI Broth) were the culture media employed.

Microorganism inoculum

The bacteria had previously been cultured in sterilized BHI broth and maintained there for 24 hours at a temperature of 37 °C. Following this time, the suspension was introduced to culture using the streak depletion technique on petri dishes that included sterile Mueller Hinton Agar and cultured for an additional 24 hours. For the delivery of 0.5 McFarland turbidity (1 x 108 CFU/mL), small quantities of the produced bacterial suspension were collected, injected, and blended in a tube that included sterile saline. The administration was then validated with the use of a tubi-dimeter.

Antibacterial activity testing

The underlying idea behind the disk-diffusion approach is to apply a paper filter to the agar that has been saturated with bacterial solution in various concentrations. 6mm diameter discs with the following test material concentrations were used for this: 2000, 1000, 500, 250, and 125 µg/mL; distilled water was employed in a volume of 10 µL of solvent. Also used as a positive control were disks that included the commercially available antimicrobial (ATM) GEN - Gentamicin 10 µg (CinnaColon, Iran).

Using a sterile swab, the bacterial inoculum was put to the agar's surface and distributed over the bottom of the petri dish on four occasions at a 45-degree angle before being applied to the plate's margins and rotating the plate multiple times at a 60-degree angle. The previously sterilized disks were repositioned on the plate using sterile tweezers. The tests were completed in triplicate and stored for 24 hours at 35 °C in an oven.

Minimum Inhibitory Concentration (MIC)

Using sterile pipettes and tips, microdilution was carried out on a 96-well plate. 100µl of Mueller-Hinton broth was poured into each well. In order to dilute the test material, 100 µl of the solution was collected from the initial well and homogenized before another 100 µl was taken out for the subsequent well. All wells of lines A, B, and C underwent this procedure, yielding the following concentrations: 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.8, 3.9, 1.9, 0.9, and 0.48 µg/mL. For the common antibiotic, same procedure was carried out in well E. The next step was to introduce 10 µL of bacterial inoculum. The diluent (distilled water)-only test and sterility check were carried out. 24 hours were spent with the plates in an oven set to 35°C. A 20µL reagent containing sodium resazurin (0.01% w/v) was used in the colourimetric assay (SIGMA), which produced the reading.

Antibacterial Activity Evaluation

By planting, in Müeller-Hinton Agar, 10 µL aliquots

of the dilutions equal to the MIC and two subsequently higher (2xMIC and 4xMIC), where practicable, of the material inside of the wells of the microdilution plates, the requirement of antibacterial activity was satisfied. These concentrations just above the MIC are enough to show whether the substance exhibits bactericidal or bacteriostatic activity, with the bacteriostatic impact being demonstrated as there is the growth of bacteria in the previously delimited microdilution plate wells. Following planting, the plates will spend 24 hours in a bacteriological oven at 37 °C. The lowest concentration that prohibits observable bacterial growth or permits the production of a maximum of three Colony Forming Units (CFU) will be referred to as the Minimum Bactericidal Concentration (MBC). In triplicate, the tests will be carried out.

Statistic evaluation

Three duplicates of each experiment were carried out. With the aid of the GraphPad Prism® 5.0 program (GraphPad Software, San Diego), the data were authorized for statistical analysis. The acquired data were reported as mean + the standard deviation after being submitted for examination of variance (ANOVA). When $p < 0.05$, differences were computed and assessed using the paired t-test.

RESULTS

Screening for bacteria

None of the bacterial strains utilized in the research showed any evidence of the development of a microbial development inhibition halo during the process of testing for antibacterial activity (Table 1). Gentamicin was utilized as a method control, and it was shown that the strains of S. aureus ATCC 25923, E. coli ATCC 25922, P. mirabilis ATCC 25933, and P. aeruginosa ATCC 27853 formed growth inhibition zones in amounts of 22mm, 17mm, 20mm, and 22mm, respectively. As a negative control, a solution containing DMSO and Tween 80 was employed; no halo formation of microbial growth suppression was seen in this solution.

Minimum inhibitory concentration

The *Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853* strains showed some sensitivity vis-à-vis the myorelaxant, when they drank the testicles of microdilution, with a minimum inhibitory concentration of 500 µg/mL and 1000 µg/mL, respectively. The results can be seen in Table 2. The findings of the other isolates in the microdilution test for *P. mirabilis and S. aureus* are shown in Table 2. This compound redox reaction was seen in every well that was going to receive the test medication, suggesting that the bacterium was active.

Antibacterial action characterization

Following microdilution experiments, the antibacterial activity of Thiocolchicosidum was characterized by the drug-sensitive microorganisms P. aeruginosa and E. coli. This was accomplished by seeding aliquots of the wells according to the amounts of MIC, 2 x MIC, and 4 x MIC (which is restricted to the maximum concentration of 1,000 µg/mL) and incubating them for 24 hours under optimum conditions. As a result, it was discovered that bacterial growth was plentiful in the culture media on the plate at all doses tested, indicating that the test substance's antibacterial activity was bacteriostatic (Table 2).

DISCUSSION

A global public health issue that is having an impact on the economy and healthcare systems is the rise of MDR infections (16). Mismanagement of antibiotics has led to the use of very toxic compounds with limited therapeutic indices, which has affected not only the health sector but also agriculture, livestock, and the pharmaceutical business (17). For this reason, many techniques have been put into place, such as figuring out the proper dose for patients using TDM, finding antibiotics in food (mostly in chicken, meat, milk, and honey), and quantifying pharmaceutical company effluents (18).

Myorelaxant Thiocolchicosidum, which is produced using alkaloid colchicine, has the potential to be repositioned pharmacologically, notably in cancer (19). Using the downregulation of the NF-κB pathway and its associated gene products, investigations reveal a capacity to inhibit osteoclast-genesis produced by cancers of the breast and multiple myeloma cell lines as well as an anticancer impact (20).

There haven't been any bacteriology studies that look at the potential of Thiocolchicosidum, however, as of yet (21). It's intriguing to look at this drug's potential against germs since its safety in therapeutic use is well known. Due to the widespread usage of bacterial illnesses in modern society, particularly in less developed nations, antibiotic use is on the rise and sometimes pointless, leading to the development of bacterial resistance (22).

Acetylsalicylic acid, also known as ASA and fluoxetine are two medications with antibacterial effects that have been studied in scientific research (23). According to researchers, the in vitro antimicrobial activity of AAS results from a reduction in bacterial polysaccharide formation, which has an impact on the proliferation of these microorganisms (24). Fluoxetine inhibits conventional as well as resistant isolates of S. aureus at concentrations of 256 and 102 µg/mL in vitro, respectively. The MIC of fluoxetine against both susceptible and resistant isolates of P. aeruginosa was 161µg/mL, while against E. coli, it was 102 µg/mL

Table 1. The diameter of the zones where bacterial growth was inhibited by Thiocolchicosidum, gentamicin, and control strains Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, and Staphylococcus aureus.

Diameter of the Growth Inhibition Halo (mm)							
Strains	**Thiocolchicosidum (µg/mL)**					**Gentamicin**	***C**
	2000	**1000**	**500**	**250**	**125**	**30 µg**	
***Staphylococcus aureus* ATCC 25923**	U#	U#	U#	U#	U#	22	U#
***Escherichia coli* ATCC 25922**	U#	U#	U#	U#	U#	17	U#
***Proteus mirabilis* ATCC 25933**	U#	U#	U#	U#	U#	20	U#
***Pseudomonas aeruginosa* ATCC 27853**	U#	U#	U#	U#	U#	22	U#

***C – solvent/diluent control: Discs impregnated with a solution of DMSO (10%) and Tween 80 (2%); #U: it was not possible to visualize the formation of a halo of inhibition of bacterial growth at the concentration of the substance used in the dif-disc method.**

Table 2. Thiocolchicosidum and gentamicin›s MIC and MBC values for the strains of Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, and Proteus mirabilis, respectively.

Strains	**Thiocolchicosidum (µg/mL)**			**Gentamicin (µg/mL)**	***C1**	****C2**	*****C3**
	MIC	Effect	MBC	MIC			
***Staphylococcus aureus* ATCC 25923**	+	U#	U#	<1	+	-	+
***Escherichia coli* ATCC 25922**	500	Bacteriostatic	U#	1	+	-	+
***Proteus mirabilis* ATCC 25933**	+	U#	U#	16	+	-	+
***Pseudomonas aeruginosa* ATCC 27853**	1000	Bacteriostatic	U#	<1	+	-	+

***C1 – microbial growth control: wells containing mueller-hinton broth and bacterial inoculum, in the absence of DMSO (10%), Tween 80 (2%), thiocolchicoside or gentamicina; **C2: Culture medium sterility control: wells containing mueller-hinton broth, in the absence of bacterial inoculum, DMSO (10%), Tween 80 (2%), thiocolchicoside or gentamicina; ***C3 – solvent/diluent control: wells containing mueller-hinton broth, DMSO (10%), Tween 80 (2%) and bacterial inoculum, in the absence of thiocolchicoside or gentamicina; #U: Indeterminate for thiocolchicoside concentrations used in the assay; (-): inhibition of bacterial growth; (+): presence of bacterial growth; MIC: Minimum Inhibitory Concentration; CBM: Minimum Bactericidal Concentration.**

(25).

The information gathered during this inquiry sheds light on the use of muscle relaxants in the context of antibiotic treatment (26). Thiocolchicosidum has not previously been documented in the literature in relation to strains of bacteria in vitro, and this investigation showed a mild antibacterial effect against the strains under examination (27, 28). Thus, from the perspective of repositioning pharmaceuticals for novel therapeutics against infectious pathogens, these data will direct future research (29, 30).

Despite having cytotoxic effects, this medication had no discernible antibacterial effects on the ATCC strains utilized in the study. More research is still required to determine how this medication affects other microbes, including fungus, protozoa, and other bacterial species.

Acknowledgements

The authors would like to thank the staff members of the Biotechnology Research Center of the Parsian BioProducts Company (PBP) in Iran. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data generated or analyzed during this study are included in this article.

REFERENCES

1.Garzón V, Bustos RH, G. Pinacho D. Personalized medicine for antibiotics: the role of nanobiosensors in therapeutic drug monitoring. Journal of Personalized Medicine. 2020;

Sep 25;10(4):147.
2.Arredondo A, Blanc V, Mor C, Nart J, León R. Resistance to β-lactams and distribution of β-lactam resistance genes in subgingival microbiota from Spanish patients with periodontitis. Clinical oral investigations. 2020; Dec;24:4639-48.
3.Chang HH, Cohen T, Grad YH, Hanage WP, O'Brien TF, Lipsitch M. Origin and proliferation of multiple-drug resistance in bacterial pathogens. Microbiology and Molecular Biology Reviews. 2015 ;Mar;79(1):101-16.
4.O'Neill J. Review on antimicrobial resistance. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. 2014 ;Dec 11;2014(4).
5.Asadipour E, Asgari M, Mousavi P, piri gharaghie T, Ghajari G, Mirzaei A. Nano-Biotechnology and challenges of drug delivery system in cancer treatment pathway. Chemistry & Biodiversity. 2023; Mar 1:e202201072.
6.Taghiloo S, Ghajari G, Zand Z, Kabiri-Samani S, Kabiri H, Rajaei N, Piri-Gharaghie T. Designing Alginate/Chitosan Nanoparticles Containing Echinacea angustifolia: A Novel Candidate For Combating Multidrug-resistant Staphylococcus aureus. Chemistry & Biodiversity. 2023; May 9:e202201008.
7.Piri Gharaghie T, Doosti A, Mirzaei SA. Prevalence and antibiotic resistance pattern of Acinetobacter spp. infections in Shahrekord medical centers. Developmental Biology. 2021 ;Nov 22;13(4):35-46.
8.Piri Gharaghie T, Sadat Shandiz SA, Beiranvand S. Evaluation of silver nanoparticles effects on bla-per1 gene expression for biofilm formation in isolates of antibiotic-resistant Acientobacter Bumanni by real time PCR method. Cellular and Molecular Research (Iranian Journal of Biology). 2022 ;Jun 22;35(2):349-66.
9.Reeves D, Lovering A, Thomson A. Therapeutic drug monitoring in the past 40 years of the Journal of Antimicrobial Chemotherapy. Journal of Antimicrobial Chemotherapy. 2016; Dec 1;71(12):3330-2.
10.Piri Gharaghie T, Sadat Shandiz SA. The inhibitory effects of silver nanoparticles on bap gene expression in antibiotic-resistant acientobacter bumanni isolates using real-time PCR. Journal of Ilam University of Medical Sciences. 2018 ;Nov 15;26(4):175-85.
11.Garzón V, Pinacho DG, Bustos RH, Garzón G, Bustamante S. Optical biosensors for therapeutic drug monitoring. Biosensors. 2019 ;Nov 11;9(4):132.
12.Mabilat C, Gros MF, Nicolau D, Mouton JW, Textoris J, Roberts JA, Cotta MO, van Belkum A, Caniaux I. Diagnostic and medical needs for therapeutic drug monitoring of antibiotics. European Journal of Clinical Microbiology & Infectious Diseases. 2020; May;39:791-7.
13.Fadeel B, Garcia-Bennett AE. Better safe than sorry: Understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. Advanced drug delivery reviews. 2010 Mar 8;62(3):362-74.
14.Hoyos P, Pace V, Alcántara AR. Biocatalyzed synthesis of statins: A sustainable strategy for the preparation of valuable drugs. Catalysts. 2019; Mar 14;9(3):260.
15.Bastos ML, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, Lan Z, Law S, MacLean E, Trajman A, Menzies D. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. bmj. 2020 Jul 1;370.
16.Majumder MA, Rahman S, Cohall D, Bharatha A, Singh K, Haque M, Gittens-St Hilaire M. Antimicrobial stewardship: Fighting antimicrobial resistance and protecting global public health. Infection and drug resistance. 2020; Dec 29:4713-38.
17.Ghajari G, Nabiuni M, Amini E. The association between testicular toxicity induced by Li2Co3 and protective effect of Ganoderma lucidum: Alteration of Bax & c-Kit genes expression. Tissue and Cell. 2021; Oct 1;72:101552.
18.Ghimpețeanu OM, Pogurschi EN, Popa DC, Dragomir N, Drăgotoiu T, Mihai OD, Petcu CD. Antibiotic use in livestock and residues in food—A public health threat: A review. Foods. 2022; May 16;11(10):1430.
19.Gracheva IA, Shchegravina ES, Schmalz HG, Beletskaya IP, Fedorov AY. Colchicine alkaloids and synthetic analogues: current progress and perspectives. Journal of medicinal chemistry. 2020; May 20;63(19):10618-51.
20.Alhmied F, Alammar A, Alsultan B, Alshehri M, Pottoo FH. Molecular mechanisms of thymoquinone as anticancer agent. Combinatorial Chemistry & High Throughput Screening. 2021; Nov 1;24(10):1644-53.
21.Souod N, Kargar M, Doosti A, Ranjbar R, Sarshar M. Genetic analysis of cagA and vacA genes in Helicobacter pylori isolates and their relationship with gastroduodenal diseases in the west of Iran. Iranian Red Crescent Medical Journal. 2013; May;15(5):371.
22.Azadbakht N, Doosti A, Jami MS. CRISPR/Cas9-mediated LINC00511 knockout strategies, increased apoptosis of breast cancer cells via suppressing antiapoptotic genes. Biological procedures online. 2022 ;Dec;24(1):1-5.
23.Raghunath D. Emerging antibiotic resistance in bacteria with special reference to India. Journal of biosciences. 2008 ;Nov;33(4):593-603.
24.Hadera M, Mehari S, Basha NS, Amha ND, Berhane Y. Study on antimicrobial potential of selected non-antibiotics and its interaction with conventional antibiotics. Pharmaceutical and Biosciences Journal. 2018 ; Jan 23:01-7.
25.Tong Z, Zhang L, Ling J, Jian Y, Huang L, Deng D. An in vitro study on the effect of free amino acids alone or in combination with nisin on biofilms as well as on planktonic bacteria of Streptococcus mutans. PloS one. 2014 ; Jun 17;9(6):e99513.
26.Kargar M, Mohammadalipour Z, Doosti A, Lorzadeh S, Japoni-Nejad A. High prevalence of class 1 to 3 integrons among multidrug-resistant diarrheagenic Escherichia coli in southwest of Iran. Osong public health and research perspectives. 2014; Aug 1;5(4):193-8.
27.Ortega-Rojas MA, Castillo E, Razo-Hernández RS, Pastor

N, Juaristi E, Escalante J. Effect of the Substituent and Amino Group Position on the Lipase-Catalyzed Resolution of γ-Amino Esters: A Molecular Docking Study Shedding Light on Candida antarctica lipase B Enantioselectivity. European Journal of Organic Chemistry. 2021 Sep 14;2021; (34):4790-802.

28.Huczyński A, Rutkowski J, Popiel K, Maj E, Wietrzyk J, Stefańska J, Majcher U, Bartl F. Synthesis, antiproliferative and antibacterial evaluation of C-ring modified colchicine analogues. European Journal of Medicinal Chemistry. 2015; Jan 27;90:296-301.

29.Zhang Z, Zhou L, Xie N, Nice EC, Zhang T, Cui Y, Huang C. Overcoming cancer therapeutic bottleneck by drug repurposing. Signal transduction and targeted therapy. 2020 Jul 2;5(1):113.

30.Gupta SC, Sung B, Prasad S, Webb LJ, Aggarwal BB. Cancer drug discovery by repurposing: teaching new tricks to old dogs. Trends in pharmacological sciences. 2013 ;Sep 1;34(9):508-17.

http://pmjournal.ir Case Study

Spring 2023, Volume 8, Issue 29 (31-35)

Correlation between Inflammatory Aortic Disease and Rheumatoid Arthritis Based on Personalized Medicine

Babak Farshid[1]; Hamidreza Kabiri[2]*

[1]*Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.*
[2] *Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.*

DOI: 10.22034/pmj.2023.705461

**Corresponding author: Hamidreza Kabiri, Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. Email: sabaco92@yahoo.com.*

Submitted: 2023-03-15
Accepted: 2023-05-29

Keywords:
Rheumatoid arthritis
Rheumatoid vasculitis
Inflammatory Aortic Disease

Abstract:
The synovial joints are most affected by the systemic autoimmune inflammatory condition known as rheumatoid arthritis (RA). A unique and underappreciated link exists between rheumatoid vasculitis (RV), an extra-articular symptom of RA, and Inflammatory Aortic Disease (aortitis). In this article, we describe the case of a 64-year-old lady who had RA-associated aortitis and conducted a literature search on the condition. Patients with RA-associated aortitis received an average oral steroid dosage of 40.2 mg/day of prednisolone (PSL). Due to the patient's RV-related symptoms, including epidermal ulceration, a significant rheumatoid factor titer, and a modest PSL dosage significantly alleviated the clinical features, it was assumed that RV also caused our patient's aortitis. Early identification and the start of therapy are crucial since RA-associated aortitis may be lethal if left untreated.

INTRODUCTION

The aortic wall becomes inflamed when Inflammatory Aortic Disease (aortitis) occurs (1). The condition is uncommon and perhaps fatal. According to statistics, there are only 1-3 new instances of aortitis per million individuals per year in the United States and Europe. Ages 10 to 40 are the most prevalent in which aortitis occurs (1, 2).

Aortitis is an inflammation that attacks major arteries more frequently than other vasculitides. Ankylosing spondylitis (AS), Behçet›s illness, rheumatic diseases (RA), and lupus erythematosus systemic are autoimmune conditions linked to aortitis. Additionally, infectious disorders like syphilis and TB are linked to aortitis (3). Inflammation is a common factor in the causes of both rheumatic and heart diseases. The elevated concentrations of inflammatory response that classify rheumatic illnesses offer a «natural experiment» to help understand the processes by which inflammatory response expedites heart disease (4). The most prevalent rheumatic disease, rheumatoid arthritis (RA), has the most thoroughly researched associations with heart disease (5, 6).

An auto - immune and structural inflammatory condition, rheumatoid arthritis primarily affects the synovial fluid. RA patients can have extra-articular symptoms such rheumatoid vasculitis (RV). Once vasculitis with major clinical symptoms was first recorded in RA patients in the 1960s, the idea of RV began to take shape. Around 90percent of RA patients with RV show skin symptoms; other clinical signs of vasculitis involve weakness, weight loss, skin irritation, cutaneous bleeding ulcers, necrosis, neuropathic pain, and abdominal infarction (7, 8).

The risk of cardiovascular disease (CVD) is increased by rheumatoid arthritis (RA). In RA patients, atherosclerosis progresses more quickly, increasing mortality. Chronic systemic inflammation may hasten the progression of atherosclerosis in RA patients due to the exact pathophysiological mechanisms between an inflammatory synovial membrane and an atherosclerotic plaque. Previous research has shown that atherosclerosis is more common in the RA group than in the general population (9, 10). RA patients' joints may be assessed with 18F-fluorodeoxyglucose (FDG) positron emission (PET)/computed tomography (CT). Since the 18F-FDG absorption represents the glucose production of monocytes in atherosclerotic plaque, FDG PET/CT has also been frequently utilized to assess atherosclerosis. FDG PET/CT is a very reliable technique for detecting arterial inflammation, however, there are few publications on how well it works to evaluate aortic inflammatory response in RA patients (9, 10).

Aortitis and RV rarely coexist, and this connection is not well known. A researcher first identified RA aortitis, and earlier studies have noted it. According to Miller et al., 45 incidents of active non-infectious aortitis were observed between 513 successive ascending aortic resections, and 4% of those patients had RA (11, 12). In this work, we report a case of RA-related aortitis and conduct a systematic review.

CASE DESCRIPTION

Patient details

We described a case of RA-related aortitis and examined 31 cases from 10 papers published on the condition. We searched PubMed for reported situations of RA aortitis using the words "osteoarthritis," "autoimmune disease," "aortitis," and "portal vein vasculitis." We assessed pathology, laboratory results, human lymphocytes antigen (HLA) pattern, age at RA and aortitis emergence, the time between RA and aortitis emergence, and other clinical findings.

Diagnostic assessment

A 64-year-old lady who had been experiencing discomfort and inflammation in the tiny bones of her arms for the previous two months went to the hospital. The experimental test results showed elevated levels of C-reactive protein (CRP; 1.91 mg/L) and erythrocyte sedimentation rate (ESR; 29 mm/h). Her anti-cyclic citrullinated protein (anti-CCP) amount was 57.3 IU/mL, and her inflammatory arthritis factor (RF) amount was 238.1 IU/mL. The nonspecific interlayer pneumonia (NSIP) sequence on the subsequent radiograph indicated interlayer pneumonia, which was provocative of RA. The patient was identified as having RA based on these findings. Salazosulfapyridine (SASP) at 750 mg/day was used to treat her RA, which helped with her clinical signs and test results. However, according to the clinical lesson, SASP was found to be insufficiently efficient after a year of treatment; as a result, 3 mg/day of tacrolimus was added to the regimen. Her articular signs and the inflammatory research results in the lab subsided after that.

After receiving treatment, she visited an outpatient facility with a 38–39°C fever, a cough, and top back problems that had been prevalent for a week. Additionally, a month earlier, she had established a skin ulcer on her right patella. During the sample analysis, inflammatory mediators with elevated levels were discovered to be ESR 108 mm/h and CRP 15.04 mg/dL. Her rheumatoid factor (RF) and matrix metalloproteinase-3 (MMP-3) amounts were 124.6 IU/L and 2,071.0 IU/mL, respectively. The C3 and C4 levels are decreased to 122.0 and 12.7 mg/dL, respectively. Antibodies against nuclear, DNA, Sm, RNP, and La were all negative. Antinuclear antibodies, perinuclear anti-neutrophil cytoplasmic antibodies (C-ANCA), and cytoplasmic pattern anti-neutrophil cytoplasmic antibodies (C-ANCA) were all negative. Three various sets of clinical specimens came back empty. A negative result was obtained for the galactomannan antigen, -D-glucan, QuantiFERON test, rapid plasma reagin test (RPR), and Treponema pallidum haemagglutination assay (TPHA). An echocardiogram revealed no indications of vegetation in her heart. A thoracic-abdominal computed tomography (CT) was performed to investigate the persistently elevated inflammatory markers. The results showed no signs of pneumonia but that the left subclavian artery, brachiocephalic artery, ascending and descending aorta, and aortic arch were all dilated. Atherosclerosis, aneurysms, or any risk variables for bacterial aneurysms were also not present. Fluorodeoxyglucose-positron emission tomography/CT (FDG-PET/CT) later demonstrated a significant abnormal tracer uptake consistent with the CT-detected dilated aortas (Figure 1A). Additionally, FDG uptake was seen in the knee skin ulcer (Figure 1B). Despite having the ulcer's skin biopsied, no clear vasculitis findings were found (Figure 1C).

Antibacterial therapy was first started, but it did not affect the fever, the elevated ESR and CRP, or the radiographic evidence of aortic dilation. Prednisolone [PSL], 30 mg/day) was started seven days after the antibiotic therapy because we suspected autoimmune aortitis. One day after beginning steroid medication, the patient's fever subsided, she no longer had upper back discomfort, and the inflammatory markers started to fall.

The skin ulcer on the right knee also significantly improved. Within a month of beginning the steroid therapy, these abnormalities returned to normal. Following the start of steroid therapy, on day 28, a follow-up CT revealed that the aortic dilation was gone entirely (Figure 2). Additionally, on day 42 following the start of the steroid treatment, FDG-PET showed that the aorta's abnormally high level of intense tracer uptake had also stopped (Figure 2). The patient was released after the prednisolone dosage was decreased to 27.5 mg daily.

Overview of relevant literature and evaluation of our case

We looked at 31 scenarios from 10 RA aortitis studies and found one instance of aortitis that was RA-related. The patients' average ages at the time of RA and aortitis diagnoses were 48±10.2 and 51±11.3, respectively. The median time between RA onset and aortitis's appearance was 6.0–7.07 years. Extra aortic arteritis involvement was found in 10/25 cases (53.1%).

According to the pathological results of RA aortitis, granulation tissue, granulocytes, and atherosclerosis were discovered in 6/14 patients, 3/14 cases, and 14/18 points, respectively. Regarding the laboratory results,

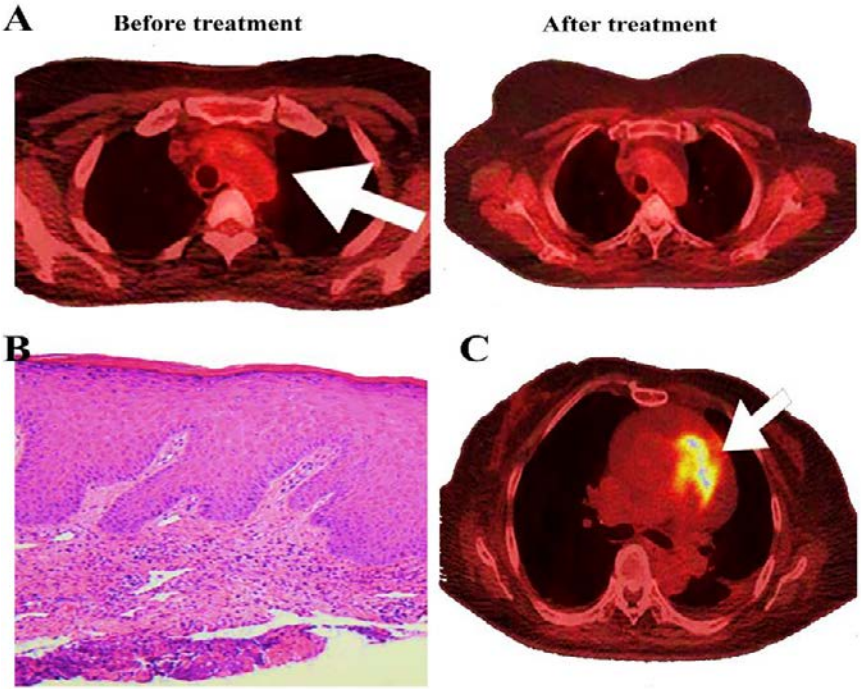


Fig1. Pathological and PET/CT results of the epidermal ulceration; FDG-PET/CT results of aortitis prior and during steroid treatment. A) The left subclavian artery, brachiocephalic artery, ascending aorta, and aortic arch showed enhanced tracer absorption on FDG PET/CT. B) A minor lymphocytic infiltration was seen in the superficial epidermis after a biopsy of the ulcer›s margin, but no overt signs of vasculitis were found. C) An ulcer with enhanced tracer absorption on FDG PET/CT suggested inflammation.

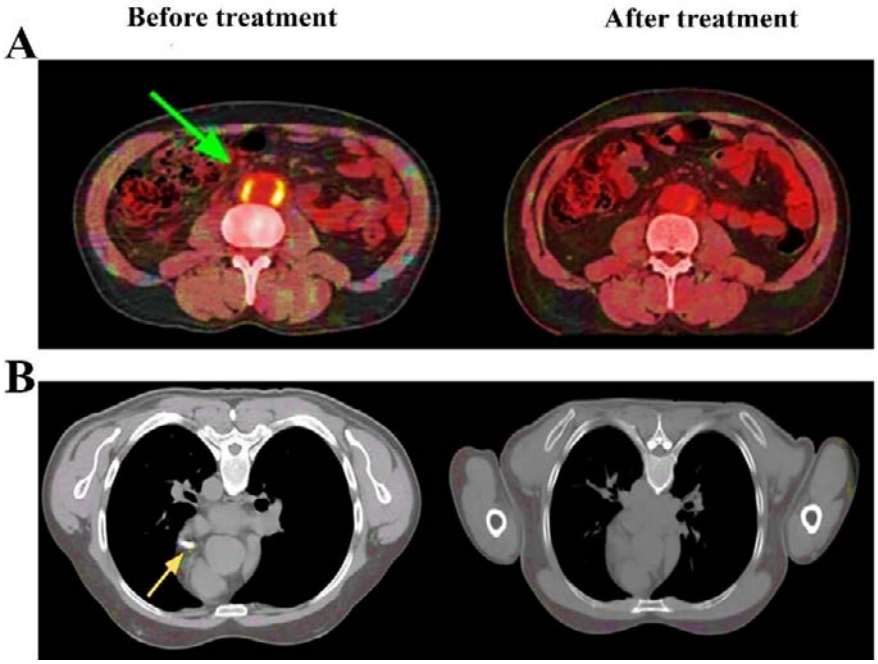


Fig2. Aortitis in the thorax as shown on contrast-enhanced CT prior to and following steroid treatment. A) Thoracic especially in comparison CT imaging showed thickness of the vessel walls at the region of the aortic arch and aortic arch, left anterior artery, and left descending aorta artery prior to steroid medication. B) The swelling of the aorta wall has totally vanished after steroid medication.

RF was positive in 20/23 patients, and rheumatoid lesions were discovered in 11/21 instances. In the individuals diagnosed whose doses were disclosed, the average oral steroid dosage for aortitis was 49.1 mg/day PSL. Two patients received intravenous steroids in addition. One individual had a single round of steroid pulse medication with methyl PSL 250 mg followed by a post-treatment PSL dosage of 40 mg. Another patient received intravenous PSL100 mg and intravenous cyclophosphamide (IVCY). Before the emergence of aortitis, there were many RA treatments. Our individual was older than some previously described since the

prodromal symptoms of RA and aortitis, in this case, happened at ages 60 and 63, respectively. According to other studies, at least half of the individuals had vasculitis symptoms other than aortitis, and these symptoms were indicative of a severe case of RA. Our patient experienced a cutaneous ulcer simultaneously, consistent with our findings. PSL was given as therapy at a dosage of 30 mg daily, which is equivalent to the average recorded steroid dose.

DISCUSSION

RA-related aortic abnormal cells have been documented in case reports, but they are still relatively uncommon complications. Contrarily, AS-related aortitis is well known, and early literature suggests that, at one point, it was challenging to distinguish between aortitis brought on by RA and AS (13). Although the two have related pathophysiological observations, it is relatively simple to distinguish between aortitis in RA and that in AS, depending on the medical course, laboratory findings, and radiological experiments (14). Findings in both AS and RA aortitis include lymphoplasmacytic infiltration, necrosis of the medial smooth muscle cells, and loss of elastic fibers. A rheumatoid granuloma, in contrast, is a distinctive feature that can be easily distinguished and is only present in aortitis linked to RA (15). Additionally, many cases of autoimmune aortitis are caused by TKA and GCA. According to the 2012 CHCC, it is challenging to distinguish between the pathological findings of the aorta in these two diseases (16). There have been reports of RA and TKA occurring simultaneously, but like the aortitis in AS, it is challenging to tell whether the aortitis is caused by RA or TKA based on pathology (17).

According to reports, small and medium-sized vascular vasculitis is the leading cause of RV. However, several accounts claim that RV happens in the aorta, a large channel. Individuals with severe RA are substantially more likely to have RA-associated aortitis, suggesting a link to RV. High-titer rheumatoid factors, a duration lasting a year or more significant after the commencement, bone erosions, and rheumatoid lesions have all been described as risk factors for the development of RV. We saw the first two characteristics in our case (18).

Additionally, our patient showed signs of a skin ulcer, seen in almost 90% of individuals with RV. The patient's skin biopsy, however, revealed no signs of vasculitis. This could be because the biopsy specimen was taken from a very superficial spot; vasculitis would have been seen if the higher epidermal layer had been tested (19). According to the following considerations, vasculitis may have also been the root cause of the patient's deep skin ulcer: "no findings implying a history of trauma, pressure ulcer, or infection"; "vasculitis is the primary reason presumed when a deep skin ulcer is noticed," as it was in this case; "FDGPET/CT demonstrates FDG uptakes at the ulcer location, implying inflammatory response"; and "the patient reacted well to steroid injections." The Scott and Bacon requirements for RV would be satisfied if our patient's skin ulcer were caused by vasculitis, and it is very plausible that our patient's aortitis was related to RV (20). The genetic study has identified HLA-DRB1*0401 as a potential cause for RV, and around 5% and 21% of RV individuals are homozygous or heterozygous and heterozygotes of this gene, respectively. HLA-B51 and B52 were found in our instance, but not HLA-DRB1*0401 Behçet's syndrome and TKA are linked to HLA-B51 and HLA-B52, respectively. These conditions have a connection to aortitis.

Our individual did not exhibit the related symptoms of vaginal ulcers, ocular diseases such as uveitis, or aphthous mouth ulcers. Hence Behçet's illness was not diagnosed in her case. Given that our case's aortitis started at 64 years old and that TKA often affects people under 50, it seems improbable that it was TKA. In addition, our individual had interstitial pneumonia, indicative of RA, and fulfilled the 2010 ACR/EULAR criterion (21). Clinical signs of aortitis in our individual were significantly relieved by oral PSL at a dosage of 30 mg per day. According to our research, the typical PSL dosage for treating aortitis is 46.3 mg; as a result, aortitis might be treated with a moderate PSL dosage. However, researchers reported that ten RA-associated aortitis clients died from congestive cardiac failure, aortic exploding, and acute coronary syndrome. In addition, most of these individuals were not treated for any steroid or immunosuppressive therapy because they were not identified with aortitis until after they passed. These instances indicate that RA-associated aortitis is a severe condition that, if neglected, may be deadly (22).

CONCLUSION

We described a woman with high-titer rheumatoid factor and epidermal ulceration as RV symptoms. Aortitis was also present, and a modest dosage of PSL significantly alleviated her clinical signs. Because RA aortitis may be deadly if ignored, it is crucial to identify and treat it early.

REFERENCES

1.Marvisi C, Buttini EA, Vaglio A. Aortitis and periaortitis: the puzzling spectrum of inflammatory aortic diseases. La Presse Médicale. 2020 ;Apr 1;49(1):104018.

2.Piri-Gharaghie T, Doosti A, Mirzaei SA. Identification of antigenic properties of Acinetobacter baumannii proteins as novel putative vaccine candidates using reverse vaccinology approach. Applied Biochemistry and

Biotechnology. 2022; Oct;194(10):4892-914.
3.Crowson CS, Liao KP, Davis III JM, Solomon DH, Matteson EL, Knutson KL, Hlatky MA, Gabriel SE. Rheumatoid arthritis and cardiovascular disease. American heart journal. 2013; Oct 1;166(4):622-8.
4.Trzeciak P, Herbet M, Dudka J. Common Factors of Alzheimer's Disease and Rheumatoid Arthritis—Pathomechanism and Treatment. Molecules. 2021; Oct 5;26(19):6038.
5.Zarinnezhad A, Shahhoseini MH, Piri Gharaghie T. Evaluating the relative frequency of fungal infections in the serum of patients with multiple sclerosis and healthy subjects using PCR. Biological Journal of Microorganism. 2021; Mar 21;10(37):37-50.
6.Piri Gharaghie T, Sadat Shandiz SA, Beiranvand S. Evaluation of silver nanoparticles effects on bla-per1 gene expression for biofilm formation in isolates of antibiotic-resistant Acientobacter Bumanni by real time PCR method. Cellular and Molecular Research (Iranian Journal of Biology). 2022 ;Jun 22;35(2):349-66.
7.Bauer ME. Accelerated immunosenescence in rheumatoid arthritis: impact on clinical progression. Immunity & Ageing. 2020 ;Dec;17(1):1-4.
8.Piri Gharaghie T, Sadat Shandiz SA. The inhibitory effects of silver nanoparticles on bap gene expression in antibiotic-resistant acientobacter bumanni isolates using real-time PCR. Journal of Ilam University of Medical Sciences. 2018; Nov 15;26(4):175-85.
9.Trang DA, Okamura K, Suto T, Sakane H, Yonemoto Y, Nakajima T, Tsushima Y, Chikuda H. Do biologic therapies reduce aortic inflammation in rheumatoid arthritis patients?. Arthritis Research & Therapy. 2021; Dec;23(1):1-7.
10.Piri-Gharaghie T. Polycystic ovary syndrome and genetic factors influencing its development: A review article. Personalized Medicine Journal. 2021; Dec 1;6(23):25-9.
11.Miller DV, Isotalo PA, Weyand CM, Edwards WD, Aubry MC, Tazelaar HD. Surgical pathology of noninfectious ascending aortitis: a study of 45 cases with emphasis on an isolated variant. The American journal of surgical pathology. 2006; Sep 1;30(9):1150-8.
12.Beiranvand S, Doosti A, Mirzaei SA. Putative novel B-cell vaccine candidates identified by reverse vaccinology and genomics approaches to control Acinetobacter baumannii serotypes. Infection, Genetics and Evolution. 2021; Dec 1;96:105138.
13.García-Martínez A, Prieto-González S, Giménez PA, Espígol-Frigolé G, Hernández-Rodríguez J, Cid MC. Aortitis and aortic aneurysm in systemic vasculitis. InEtiology, Pathogenesis and Pathophysiology of Aortic Aneurysms and Aneurysm Rupture 2011; Jul 27. IntechOpen.
14.Ferrera C, Vilacosta I, Cabeza B, Cobiella J, Martínez I, Saiz-Pardo Sanz M, Bustos A, Serrano FJ, Maroto L. Diagnosing aortic intramural hematoma: current perspectives. Vascular Health and Risk Management. 2020; Jun 8:203-13.
15.Töpel I, Zorger N, Steinbauer M. Inflammatory diseases of the aorta: Part 1: non-infectious aortitis. Gefasschirurgie. 2016;21(Suppl 2):80.
16.Hur JH, Chun EJ, Kwag HJ, Yoo JY, Kim HY, Kim JJ, Lee KW. CT features of vasculitides based on the 2012 International Chapel Hill Consensus Conference revised classification. Korean Journal of Radiology. 2017; Oct 1;18(5):786-98.
17.Moittié S, Sheppard MN, Thiele T, Baiker K. Non-Infectious, Necrotizing and Granulomatous Aortitis in a Female Gorilla. Journal of Comparative Pathology. 2020; Nov 1;181:7-12.
18.Nikol S, Mathias K, Olinic DM, Blinc A, Espinola-Klein C. Aneurysms and dissections-What is new in the literature of 2019/2020-a European Society of Vascular Medicine annual review. VASA. 2020; Aug 27.
19.Sangolli PM, Lakshmi DV. Vasculitis: A checklist to approach and treatment update for dermatologists. Indian dermatology online journal. 2019; Nov;10(6):617.
20.Quintana-Castanedo L, Feito-Rodríguez M, Fernández-Alcalde C, Granados-Fernández M, Montero-Vega D, Mayor-Ibarguren A, de Lucas-Laguna R. Concurrent chilblains and retinal vasculitis in a child with COVID-19. Journal of the European Academy of Dermatology and Venereology. 2020; Dec;34(12):e764.
21.Algahtani H, Shirah B, Khafaji R, Algahtani S. Novel Heterozygous Variants in the HLA-DRB1 Gene in a Saudi Family With Early-Onset Familial Multiple Sclerosis: Therapeutic Failure and Success. International Journal of MS Care. 2022; May 1;24(3):100-3.
22.Akebo H, Sada R, Matsushita S, Ishimaru H, Minoda S, Miyake H, Tsugihashi Y, Hatta K. Lupus aortitis successfully treated with moderate-dose glucocorticoids: a case report and review of the literature. Internal Medicine. 2020; Nov 1;59(21):2789-95.

http://pmjournal.ir Reviwe Article

Spring 2023, Volume 8, Issue 29 (36-42)

The Connection between Platelets and the Development of Cancer

Golnaz Zamanian[1*], Peyman Ghoraishizadeh[2], Sajad Hossein Bazrafshani[3]

[1] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[2] Department of Clinical Microscopy, Faculty of Medical Technology, Mahidol University, Bangkok, 10700, Thailand.
[3] E.N.T. Department, Worcester Royal Hospital, Worcester, UK.

DOI: 10.22034/pmj.2023.705458

**Corresponding author: Golnaz Zamanian, Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran. Email: golnazzamanian@gmail.com*

Submitted: 2023-03-02
Accepted: 2023-05-26

Keywords:
Cancer
Tumor
Metastatic
Platelets

Abstract:
Platelets are tiny (2-4 m), anucleate, hematopoietic cells that are discharged into the circulation by bone marrow megakaryocytes. Platelets were formerly thought to be the main agents of hemostasis and thrombosis. Armand Trousseau established a strong link between thrombosis and cancer in 1865. The hypothesis that platelets play many roles in the development of malignancies and in cancer-associated thrombosis is thus supported by a wealth of clinical and experimental data.The functions of tumor-educated platelets (TEPs) in the development of cancer, from primary tumors to subsequent metastatic breakouts, will be covered in this study.

INTRODUCTION

Platelets are tiny (2-4 m) anucleate hematopoietic cells that are discharged into the circulation by bone marrow megakaryocytes. Circulating platelets in healthy people range in number from 150 to 350 109/L. It was recently shown in a mouse model that intravascular megakaryocytes originating from extrapulmonary locations may also manufacture platelets in the lung (1). Platelets were formerly thought to be the main agents of hemostasis and thrombosis. Osler, who demonstrated the existence of "blood plaques" in white thrombi, provided the first description of the hemostatic properties of platelets in 1873. Armand Trousseau established a strong link between thrombosis and cancer in 1865 (2). The hypothesis that platelets play many roles in the development of malignancies and in cancer-associated thrombosis is thus supported by a large body of experimental as well as clinical data (3). The vast majority of circulating and ingested biomolecules, as well as those that are specific to platelets, are stored in the body's platelets. Upon activation, platelets release the biomolecules in their granules that aid in the development of cancer. Cancer and platelets interact in a genuine way. In fact, platelet count and activation state, which are essential for cancer progression, can be affected by cancer itself. The ability of cancer cells to increase platelet count is not unharmful, as evidenced by the relationship between inhibition of thrombocytosis or induction of thrombocytopenia and a reduction in tumour growth and metastasis (7, 8). Yet there is disagreement over platelets' functions in the development of tumours. On the one hand, a large body of research has shown that platelets promote the growth, angiogenesis, pro-survival signalling, and invasiveness of cancer cells.In ovarian cancer cells, for instance, Egan and colleagues demonstrated that platelet adherence and degranulation increased (9). Moreover, platelet stickiness and substances secreted by platelets seem to actively contribute to the epithelial-to-mesenchymal transformation (EMT) of cancer cells, increasing their capacity for invasion and metastasis (10). Nonetheless, a few studies have shown that platelets or platelet-derived microparticles have an anti-proliferative effect on cancer cells, as shown by the activation of cell cycle arrest, inhibition of DNA synthesis, and triggering of apoptosis, respectively (11, 12). Platelets may also be "taught" by cancer cells by altering their RNA patterns and morphologies. Many cancer forms, particularly lung, prostate, glioma, and breast carcinoma, have been linked to altered platelet RNA profiles (4–6). Best et al.'s accuracy in separating cancer patients from healthy people using RNA-seq data suggests that the recently developed idea of tumour-educated platelets (TEPs) may provide useful tools for cancer diagnosis (6). The functions of platelets and tumour-educated platelets (TEPs) in the development of cancer, from primary tumours to subsequent metastatic breakouts,

will be covered in this paper(13).

Growth, angiogenesis, and metastasis-promoting angiogenic agents in tumor development

Platelet alpha granules are particularly abundant in growth factors and mitogens like TGF (transforming growth factor), EGF (epidermal growth factor), and PDGF (14). PDGFR-mediated signaling by platelet-derived growth factors (PDGF) activates a variety of cellular processes, including cell division, growth, and proliferation. Metastatic breast tumors' growth is accelerated by PDGF, and a shorter survival time and higher degree of metastasis are linked to breast cancer patients' elevated serum levels of PDGF(15). VEGF, a growth factor essential in the stimulation of angiogenesis and vasculogenesis, is also present in platelet alpha granules. In 1974, Folkman and colleagues published the first description of tumor angiogenesis, showing that tumors can only grow to a diameter of 2 mm before they must sprout new blood vessels to maintain the diffusion of nutrients and oxygen needed for tumor growth(16).

VEGF-R promotes endothelial cell migration, proliferation, and vessel creation. Platelets contain a plethora of angiogenesis-regulating proteins in their granules, including VEGF, PDGF, TGF, EGF, angiopoietin-1, IGF-1, sphingosine-1-phosphate, and MMPs(17). Rong Li and colleagues showed that intratumoral platelets have a role in controlling blood vessel development and density, mostly via the production of VEGF and TGF(18). Therapeutic VEGF monoclonal antibodies and tyrosine kinase inhibitors have been developed. TGF, a mitogen produced with platelet activation from alpha granules, has been linked to tumor development, metastasis, and a poor prognosis(19).

TGF can function as a tumor suppressor and effectively stop the growth and proliferation of tumors and cancer cells in their early stages, but it is primarily thought to promote tumor growth and metastasis because platelets release it into the microenvironment. Direct contact between platelets and cancer cells and the release of platelet-derived TGF activate TGF(20).

Procoagulant proteins, hemostatic agents, and platelet agonists in tumor angiogenesis, growth, and metastasis

ADP, TXA2, and thrombin, three platelet agonists that certain cancer cells may produce, have all been demonstrated to cause platelet activation and to aid in the development and spread of tumors (21–22). Cho et al. showed that platelets promote the growth of ovarian cancer cells in a TGF-dependent way (24). Moreover, this team recently showed that ticagrelor inhibits the platelet ADP receptor P2Y12, which reduces ovarian tumor development by 60% compared to aspirin and by 75% compared to mice receiving a placebo (25).

Our research found that clopidogrel, another P2Y12 inhibitor, decreases pancreatic tumor development and metastasis but uses an orthotopic pancreatic cancer mouse model (26). Moreover, Simon Gebremeskel and associates demonstrated that ticagrelor's reversible suppression of P2Y12 reduces metastases and increases survival in a mouse model of melanoma (27). These results offer proof of concept for the therapeutic use of P2Y12 inhibitors like clopidogrel and ticagrelor for the prevention of tumor progression and metastasis and suggest a role for P2Y12-mediated platelet activation in encouraging tumor growth and metastasis. Moreover, it has been shown that the activation of the endothelium P2Y2 receptor by platelet-tumor cell interaction-dependent ATP release increases vascular permeability, promoting tumor cell extravasation and metastatic seeding (28). Since the 1980s, thromboxane, which is produced after platelet aggregation of cancer cells, has received attention and is positively correlated with the progression of ovarian cancer. Many malignancies, including colorectal, prostate, bladder, and non-small-cell lung carcinomas, have been shown to overexpress thromboxane ligands and thromboxane synthase. The addition of TXA2 restored tumor growth when thromboxane synthase function was inhibited, and it also triggered apoptosis (29, 30). Cancer cells have a number of functions in tumor development, angiogenesis, and invasion, including the production of thrombin after platelet activation, which is controlled by cancer cells, and thrombin release by cancer cells themselves. In several cancer cell lines, including human and mouse breast carcinomas, prostate carcinomas, and melanomas, as well as primary endothelial cells, commonly known as HUVEC (human umbilical vein endothelial cells), thrombin treatment increases cathepsin D (CD) mRNA and protein expression. The improvement of cancer cell chemotaxis and migration, as well as HUVEC matrigel tube formation, was caused in these cell lines by up-regulation of CD expression and secretion. Moreover, the use of CD-knockdown cancer cells and the pharmaceutical suppression of thrombin by hirudin both significantly slow tumor growth and metastasis (31, 32). Through the induction and interaction of MMP-9 and 1-integrin on the cell surface through a PI3K-dependent mechanism, thrombin also facilitates tumor invasion (33). Moreover, thrombin may cause EMT in SKOV3 ovarian cancer cells, which enhances the cells' propensity for invasion (34). Additionally, thrombin increases the production of VEGF and tissue factor (TF) by MDA-231 breast cancer cells, which increases the cells' ability to metastasize (35). A transmembrane glycoprotein called TF helps the extrinsic route of the body's regular blood coagulation protease cascade get started. To create an active complex that is in charge of thrombin production,

fibrin deposition, and the proteolytic activation of factors IX and X, TF can bind to factor VIIa (TF/fVIIa). Coagulation factor extravasation was seen in the tumor microenvironment, mostly as a result of increased tumor vascular permeability. According to research by Liu and colleagues, the TF produced by cancer cells and the TF-activated coagulation cascade in the tumor microenvironment are crucial for the development of tumors. A TF/FVIIa inhibitor caused growth retardation in mice treated with breast tumors, whereas doxorubicin-based prodrugs that are specifically activated by the protease activity of TF, FVIIa, FXa, and thrombin completely eliminated both the primary tumor and metastasis (36). Through the ligation of endothelial integrins v3 and 61, the deposit of the non-coagulant alternatively spliced isoform of TF into the tumor stroma induced angiogenesis. Additionally, this team demonstrated that inhibiting TF-VIIa-PAR2 signaling but not TF-initiated coagulation reduced the growth and angiogenesis of breast tumors (37). These studies show that TF is essential for tumor development and angiogenesis and that the protease activity of the coagulation cascade in the tumor microenvironment may act as an enzymatic target for chemotherapeutic pro-drugs. When platelets are activated, the platelet peroxisomes contain the platelet-activating factors (PAFs), which are then released. It has been shown that certain melanoma cell lines possess a functioning platelet activating factor receptor (PAF-R), which, via its signaling, initiates a pro-survival program. Additionally, it was shown that PAFs may increase VEGF expression in immortalized vascular cells, mostly via activating the NF-kappaB pathway and reducing p53 activity (38). Furthermore, blocking PAFR signaling with antagonists seems to limit tumor development and restrict angiogenesis in breast, prostate, colitis-associated cancer, and Kaposi's sarcoma (39–40).

The functions of adhesion proteins in tumor development, metastasis, and dissemination

Cancer cells have the ability to stimulate platelets in two main ways: indirectly via a variety of secreted substances and directly through their adherence to moving platelets. The development of cancer seems to depend on direct contact between platelets and cancer cells(41). First, the survival of tumor cells in the circulation depends on the collection of platelets surrounding them. In fact, it has been shown that in the lungs of mice, platelet-coated tumor cells and fibrinogen deposition create a physical barrier that shields tumor cells from the cytotoxic action of NK cells (42). Furthermore, the activating immunoreceptor NKG2D on NK cells was down-regulated as a result of platelet activation and subsequent release of platelet-derived TGF, which reduced their anti-tumor efficacy (43). Platelet aggregation surrounding cancer cells is crucial for both shielding cancer cells from severe shear stress in the circulation and thwarting immune attack (44). Second, it seems that platelet adhesion and degranulation are required for ovarian cancer cells to produce pro-survival and pro-angiogenic signals (45). Similar to this, Labelle et al. showed that the synergistic activation of TGF/smad and NF-kB pathways in breast and colorectal cancer cells depends on direct contact between platelets and cancer cells, followed by the release of platelet-derived TGF. The stimulation of these pathways causes greater metastasis in vivo and the development of an invasive mesenchymal-like phenotype in vitro(46). Additionally, it has been shown that the capacity of cancer cells to cause platelet aggregation, also known as tumor cell-induced platelet aggregation (TCIPA), and thrombocytopenia in mice is closely connected to their capacity to spread in vivo (47). Together, these findings show that interactions between platelets and cancer cells are crucial for the development of tumors. Few studies, however, have examined the function of platelet and cancer cell adhesive proteins in the development of malignancies as well as their potential as therapies (48).

Integrins

Karpatkin et al.'s research from 1988 showed that platelet integrin IIb3 suppression by blocking antibodies decreased colorectal and melanoma cancer cell-platelet interactions in vitro and decreased metastasis in vivo. It is possible that fibronectin and the Von Willebrand factor (VWF) interact in a way that is IIb3-dependent since the addition of RGDS inhibited the contacts between platelets and cancer cells (49). Furthermore, tumor cell integrin v3 has been shown to bind platelet integrin IIb3, mediating the contact and aggregation of cancer cells with platelets as well as promoting tumor development and metastasis in vivo (50–51).Platelet integrin 61 interacts directly with colorectal MC-38 and breast cancer AT3 cell ADAM9, according to research by Mammadova-Bach et al. that was published more recently. Interactions between platelet integrin 61 and tumor cells that are reliant on ADAM9 cause platelet activation, granule release, and the encouragement of cancer cell extravasation in the lungs (52).

Selectins and mucins

P-selection is a protein that facilitates the interaction of platelets with a variety of cancer cell lines, including colorectal, lung, breast, gastric, and melanomas. It is expressed on the surface of activated platelets and is implicated in tumor development and metastasis(53). Podoplanin (PDPN) and colon, bladder, and lung carcinomas have increased expression of P-selectin. P-selectin is the first mediator of the rolling and

anchoring of platelet-cancer cell aggregates to the endothelium, which is then facilitated by the platelet adhesive proteins GP1b, IIb-3 integrin, and VWF. Additional platelet receptors that some cancer cells can express include GP1ba, IIb-3 integrin, and v-3 integrin. (54).

The role of immunosuppression, platelet chemokines, and tumor development, angiogenesis, and metastasis

It seems that platelet chemokines are essential for the development of malignancy and immunosuppression. Since the early 1990s, attention has been focused on the role of platelet factor-4 (PF4), or chemokine CXCL4, in the suppression of angiogenesis. Angiostatic chemokine PF4/CXCL4 is released from platelet alpha granules during platelet activation(55).

Recombinant PF4 has been shown by Maione et al. to limit blood vessel growth in a dose-dependent manner using experiments on chicken chorioallantoic membranes(56). In 2004, Struyf and colleagues discovered a fresh, nonallelic variation of PF4/CXCL4 called PF4var/CXCL4L1 from thrombin-activated platelets. The two secreted forms of PF4var/CXCL4L1 seem to be a more powerful inhibitor of angiogenesis than PF4/CXCL4 and other an-giostatic chemokines, while only having a three amino acid difference between them (57). Furthermore, PF4var/CXCL4L1 inhibited angiogenesis more effectively than PF4/CXCL4 or other angiostatic chemokines, such as interferon gamma (IFNgamma), in animal models of melanoma and lung tumours (58-59).

Platelets actively contribute to the persistence of cancer cells and stop them on the vessel walls during the spread of cancer cells via the blood. Additionally, Labelle and associates showed in the lungs of mice that intravascular platelet-tumor cell microthrombi release the chemokine CXCL5/7 that recruits +CD11b+MMP9 +LY6G granulocyte cells to form early metastatic niches. Inhibiting CXCR2, which blocks the CXL5/7 receptor on granulocytes, prevented granulocyte recruitment and prevented metastatic seeding, proving that platelet-mediated granulocyte recruitment is essential for the development of metastatic niches (60).

Platelet microparticles (PMPs)

Megakaryocytes and platelets continuously discharge platelet microparticles (PMPs), which are associated with aggressive tumors and unfavorable clinical outcomes. Through a variety of mechanisms, cancer cells can activate platelets, causing them to change shape, degranulate, and produce PMPs(61). PMPs have the potential to carry bioactive lipids such as sphingosine 1 phosphate (S1P) and arachidonic acids (AA). In vitro treatment of endothelial cells with PMPs increased proliferation, chemotactic migration, and the formation of capillary-like tubes in HUVECs. In vivo injection of PMPs into the rat myocardium induced angiogenesis and stimulated post-ischemic revascularization(62).

PMPs also enhance angiogenesis, invasiveness, and metastasis in breast and lung malignancies. PMPs increased the mRNA expression of angiogenic factors such as VEGF, MMP-9, interleukin-8 (Il-8), and HGF in lung cancer cell lines and improved Cancer cells have a higher propensity for metastasis when they express platelet receptors, a process known as “platelet mimicry”(63). In vitro PMP administration increased the expression and secretion of membrane type-1 MMP, which is implicated in invasion behavior, in various breast and lung cancer cell lines. Additionally, PMPs have the capacity to introduce nucleic acids into cancer cells(64). Liang et al. showed that the transport of microRNA-223 into lung cancer cells by PMPs increased the invasiveness of the cancer cells(65). Recent research found that PMPs may invade solid tumors and spread miRNAs that inhibit tumor development. PMPs may operate in a way that is either pro- or anti-tumor, depending on the situation. The potential of PMPs to control immune cell activity allows them to also have an indirect impact on the development of tumors(66). Local macrophages seem to undergo a process of differentiation known as M2 macrophage differentiation, which has pro-tumorigenic properties. Sprague and colleagues investigated the function of PMPs in the control of adaptive immunity. (67).

CONCLUSION

During the development of cancer, platelets and cancer actually interact. On the one hand, a variety of platelet agonists, including ADP, thrombin, and thromboxane, are released by cancer cells. Additionally, cancer cells have the capacity to continuously produce MPs as a result of their oncogenic transformation. These MPs are released in the bloodstream and (i) take part in platelet activation and RNA profile changes, and (ii) support pro-thrombotic states in cancer patients. However, platelet activation results in the release of the active biomolecules that are present in their granules and are all involved in the development of cancer. Numerous growth and angiogenic factors found in platelets support tumor growth and angiogenesis. Additionally, platelet aggregation around tumor cells (TCIPA) provides cancer cells with a number of benefits, including the ability to evade immune surveillance, protection from shear stress, prosurvival signals, adhesion to the endothelium, and extravasation. It seems that successful metastatic breakout and tumor development depend on platelet activation. Based on these findings, several investigations should target platelet activation, sticky proteins involved in contacts between cancer cells and platelets, and alpha granule

contents to slow the evolution of tumors.

REFRENCE

1.S. Karpatkin, E. Pearlstein, C. Ambrogio, B.S. Coller, Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo, J. Clin. Invest.81. 1988 ; 1012–1019,

2.O. Engebraaten, M. Trikha, S. Juell, S. Garman-Vik, Ø. Fodstad, Inhibition of in vivo tumour growth by the blocking of host alpha(v)beta3 and alphaII(b)beta3 integrins, Anticancer Res. 29. 2009; 131–137.

3.M.R. Weber, M. Zuka, M. Lorger, M. Tschan, B.E. Torbett, A. Zijlstra, J.P. Quigley,K. Staflin, B.P. Eliceiri, J.S. Krueger, P. Marchese, Z.M. Ruggeri, B.H. Felding,Activated tumor cell integrin αvβ3 cooperates with platelets to promote extravasation and metastasis from the blood stream, Thromb. Res. 140 (Suppl.)2016 ; S27–36.

4.B. Felding-Habermann, T.E. O'Toole, J.W. Smith, E. Fransvea, Z.M. Ruggeri,M.H. Ginsberg, P.E. Hughes, N. Pampori, S.J. Shattil, A. Saven, B.M. Mueller,Integrin activation controls metastasis in human breast cancer, Proc. Natl. Acad.Sci. U. S. A. 98 .2001;1853–1858.

5.S. Mezouar, R. Darbousset, F. Dignat-George, L. Panicot-Dubois, C. Dubois,Inhibition of platelet activation prevents the P-selectin and integrin-dependent accumulation of cancer cell microparticles and reduces tumor growth and metastasis in vivo, Int. J. Cancer 136. 2015; 462–475.

6.S. Gebremeskel, T. LeVatte, R.S. Liwski, B. Johnston, M. Bezuhly, The reversible P2Y12 inhibitor ticagrelor inhibits metastasis and improves survival in mouse models of cancer, Int. J. Cancer 136 .2015; 234–240.

7.N. Fujita, S. Takagi, The impact of Aggrus/podoplanin on platelet aggregation and tumour metastasis, J. Biochem. (Tokyo) 152. 2012 407–413.

8.Y. Kato, M. Kaneko, M. Sata, N. Fujita, T. Tsuruo, M. Osawa, Enhanced expression of Aggrus (T1alpha/Podoplanin), a platelet-aggregation-inducing factor in lung squamous cell carcinoma, Tumor Biol. 26 .2005 ; 195–200.

9.S. Takagi, T. Oh-hara, S. Sato, B. Gong, M. Takami, N. Fujita, Expression of Aggrus/podoplanin in bladder cancer and its role in pulmonary metastasis, Int. J.Cancer 134.2014; 2605–2614.

10.Y. Kato, N. Fujita, A. Kunita, S. Sato, M. Kaneko, M. Osawa, T. Tsuruo, Molecular identification of Aggrus/T1alpha as a platelet aggregation-inducing factor expressed in colorectal tumors, J. Biol. Chem. 278. 2003; 51599–51605.

11.C.L. Chaffer, R.A. Weinberg, A perspective on cancer cell metastasis, Science 331.2011; 1559–1564

12.H. Läubli, L. Borsig, Selectins as mediators of lung metastasis, Cancer Microenviron. 3 .2010 ; 97–105.

13.Y. Liu, P. Jiang, K. Capkova, D. Xue, L. Ye, S.C. Sinha, N. Mackman, K.D. Janda, C. Liu, Tissue factor-activated coagulation cascade in the tumor microenvironment is critical for tumor progression and an effective target for therapy, Cancer Res. 71. 2011 ; 6492–6502,

14.W. Ruf, N. Yokota, F. Schaffner, Tissue factor in cancer progression and angio- genesis, Thromb. Res. 125 .2010; S36–S38.

15.H.-M. Ko, H.H. Jung, K.H. Seo, Y.-R. Kang, H.-A. Kim, S.J. Park, H.-K. Lee, S.-Y. Im, Platelet-activating factor-induced NF-kappaB activation enhances VEGF expression through a decrease in p53 activity, FEBS Lett. 580 (2006) 3006–3012.

16.L. Sun, Z. He, J. Ke, S. Li, X. Wu, L. Lian, X. He, X. He, J. Hu, Y. Zou, X. Wu, P. Lan, PAF receptor antagonist Ginkgolide B inhibits tumourigenesis and angiogenesis in colitis-associated cancer, Int. J. Clin. Exp. Pathol. 8 .2015; 432–440.

17. B. Bussolati, L. Biancone, P. Cassoni, S. Russo, M. Rola-Pleszczynski,G. Montrucchio, G. Camussi, PAF produced by human breast cancer cells promotes migration and proliferation of tumor cells and neo-angiogenesis, Am. J. Pathol.157 .2000; 1713–1725.

18.M. Labelle, S. Begum, R.O. Hynes, Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis, Cancer Cell 20 .2011; 576–590.

19.Y. Wang, H. Zhang, Platelet-induced inhibition of tumor cell growth, Thromb. Res. 123 .2008; 324–330.

20.J.V. Michael, J.G.T. Wurtzel, G.F. Mao, A.K. Rao, M.A. Kolpakov, A. Sabri,N.E. Hoffman, S. Rajan, D. Tomar, M. Madesh, M.T. Nieman, J. Yu, L.C. Edelstein, J.W. Rowley, A.S. Weyrich, L.E. Goldfinger, Platelet microparticles infiltrating solid tumors transfer miRNAs that suppress tumor growth, Blood 130 .2017; 567–580.

21. J.F. Di Stefano, M. Kirchner, K. Dagenhardt, N. Hagag, Activation of cancer cell proteases and cytotoxicity by EGF and PDGF growth factors, Am J Med Sci 300 .1990; 9–15.

22.J.S. Desgrosellier, D.A. Cheresh, Integrins in cancer: biological implications and therapeutic opportunities, Nat. Rev. Cancer 10 .2010; 9–22.

23.T.E. Maione, G.S. Gray, J. Petro, A.J. Hunt, A.L. Donner, S.I. Bauer, H.F. Carson, R.J. Sharpe, Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides, Science 247 .1990; 77–79.

24.S. Struyf, M.D. Burdick, P. Proost, J.V. Damme, R.M. Strieter, Platelets release CXCL4L1, a nonallelic variant of the chemokine platelet Factor-4/CXCL4 and potent inhibitor of angiogenesis, Circ. Res. 95 .2004; 855–857.

25.S. Struyf, M.D. Burdick, E. Peeters, K.V. den Broeck, C. Dillen, P. Proost,J.V. Damme, R.M. Strieter, Platelet Factor-4 variant chemokine CXCL4L1 inhibits melanoma and lung carcinoma growth and metastasis by preventing angiogenesis, Cancer Res. 67.2007; 5940–5948.

26.J. Vandercappellen, S. Liekens, A. Bronckaers, S. Noppen, I. Ronsse, C. Dillen, M. Belleri, S. Mitola, P. Proost, M. Presta, S. Struyf, J. Van Damme, The COOH- terminal peptide of platelet factor-4 variant (CXCL4L1/PF-4var47-70) strongly inhibits angiogenesis and suppresses B16 melanoma growth in vivo, Mol. Cancer Res. 8

(2010) 322–334.

27.P. Allavena, A. Sica, C. Garlanda, A. Mantovani, The yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance, Immunol. Rev. 222 (2008) 155–161.

28.G.T. Lee, J.H. Hong, C. Kwak, J. Woo, V. Liu, C. Lee, I.Y. Kim, Effect of dominant negative transforming growth factor-beta receptor type II on cytotoxic activity of RAW 264.7, a murine macrophage cell line, Cancer Res. 67 (2007) 6717–6724.

29.L. Shen, J.M. Smith, Z. Shen, M. Eriksson, C. Sentman, C.R. Wira, Inhibition of human neutrophil degranulation by transforming growth factor-β1, Clin. Exp. Immunol. 149 (2007) 155–161.

30.M. Labelle, S. Begum, R.O. Hynes, Platelets guide the formation of early metastatic niches, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) E3053–3061.

31.S. Mezouar, D. Mege, R. Darbousset, D. Farge, P. Debourdeau, F. Dignat-George, L. Panicot-Dubois, C. Dubois, Involvement of platelet-derived microparticles in tumor progression and thrombosis, Semin. Oncol. 41 (2014) 346–358.

32.C. Yang, R. Ma, T. Jiang, M. Cao, L. Zhao, Y. Bi, J. Kou, J. Shi, X. Zou, Contributions of phosphatidylserine-positive platelets and leukocytes and micro- particles to hypercoagulable state in gastric cancer patients, Tumor Biol. 37 (2016) 7881–7891.

33.A. Rank, S. Liebhardt, J. Zwirner, A. Burges, R. Nieuwland, B. Toth, Circulating microparticles in patients with benign and malignant ovarian tumors, Anticancer Res. 32 (2012) 2009–2014.

34.J.G. Ren, Q.W. Man, W. Zhang, C. Li, X.P. Xiong, J.Y. Zhu, W.M. Wang, Z.J. Sun, J. Jia, W.F. Zhang, Y.F. Zhao, G. Chen, B. Liu, Elevated level of circulating platelet- derived microparticles in oral cancer, J. Dent. Res. 95 (2016) 87–93.

35.C.-C. Tseng, C.-C. Wang, H.-C. Chang, T.-H. Tsai, L.-T. Chang, K.-T. Huang, S. Leu, C.-H. Yen, S.-F. Liu, C.-H. Chen, C.-T. Yang, H.-K. Yip, M.-C. Lin, Levels of circulating microparticles in lung cancer patients and possible prognostic value, Dis. Markers 35 (2013) 301–310.

36.L. Zhao, Y. Bi, J. Kou, J. Shi, D. Piao, Phosphatidylserine exposing-platelets and microparticles promote procoagulant activity in colon cancer patients, J. Exp. Clin. Cancer Res. 35 (2016).

37.B. Toth, S. Liebhardt, K. Steinig, N. Ditsch, A. Rank, I. Bauerfeind, M. Spannagl, K. Friese, A.J. Reininger, Platelet-derived microparticles and coagulation activation in breast cancer patients, Thromb. Haemost. 100 (2008) 663–669.

38.K. Haubold, M. Rink, B. Spath, M. Friedrich, F.K.-H. Chun, G. Marx, A. Amirkhosravi, J.L. Francis, C. Bokemeyer, B. Eifrig, F. Langer, Tissue factor procoagulant activity of plasma microparticles is increased in patients with early- stage prostate cancer, Thromb. Haemost. 101 (2009) 1147–1155.

39.F. van Doormaal, A. Kleinjan, R.J. Berckmans, N. Mackman, D. Manly,P.W. Kamphuisen, D.J. Richel, H.R. Büller, A. Sturk, R. Nieuwland, Coagulation activation and microparticle-associated coagulant activity in cancer patients, Thromb. Haemost. 108 (2012) 160–165.

40.E. Campello, L. Spiezia, C.M. Radu, C. Bulato, M. Castelli, S. Gavasso, P. Simioni, Endothelial, platelet, and tissue factor-bearing microparticles in cancer patients with and without venous thromboembolism, Thromb. Res. 127 (2011) 473–477.

41.O.P. Barry, D. Pratico, J.A. Lawson, G.A. FitzGerald, Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles, J. Clin. Invest. 99 (1997) 2118–2127.

42.S. Miyamoto, M.A. Kowalska, C. Marcinkiewicz, M.M. Marcinkiewicz, D. Mosser, L.H. Edmunds, S. Niewiarowski, Interaction of leukocytes with platelet micro- particles derived from outdated platelet concentrates, Thromb. Haemost. 80 (1998) 982–988.

43.M. Baj-Krzyworzeka, M. Majka, D. Pratico, J. Ratajczak, G. Vilaire, J. Kijowski, R. Reca, A. Janowska-Wieczorek, M.Z. Ratajczak, Platelet-derived microparticles stimulate proliferation, survival, adhesion, and chemotaxis of hematopoietic cells, Exp. Hematol. 30 (2002) 450–459.

44.H.K. Kim, K.S. Song, J.-H. Chung, K.R. Lee, S.-N. Lee, Platelet microparticles in- duce angiogenesis in vitro, Br. J. Haematol. 124 (2004) 376–384.

45.A. Janowska-Wieczorek, M. Wysoczynski, J. Kijowski, L. Marquez-Curtis,B. Machalinski, J. Ratajczak, M.Z. Ratajczak, Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer, Int. J. Cancer 113 (2005) 752–760.

46.A. Janowska-Wieczorek, L.A. Marquez-Curtis, M. Wysoczynski, M.Z. Ratajczak,Enhancing effect of platelet-derived microvesicles on the invasive potential of breast cancer cells, Transfusion (Paris) 46 (2006) 1199–1209.

47.O. Dashevsky, D. Varon, A. Brill, Platelet-derived microparticles promote inva- siveness of prostate cancer cells via upregulation of MMP-2 production, Int. J. Cancer 124 (2009).

48.H. Liang, X. Yan, Y. Pan, Y. Wang, N. Wang, L. Li, Y.

Liu, X. Chen, C.-Y. Zhang, H. Gu, K. Zen, MicroRNA-223 delivered by platelet-derived microvesicles pro- motes lung cancer cell invasion via targeting tumor suppressor EPB41L3, Mol. Cancer 14 .2015; 58.

49.A. Laios, S. O'Toole, R. Flavin, C. Martin, L. Kelly, M. Ring, S.P. Finn, C. Barrett, M. Loda, N. Gleeson, T. D'Arcy, E. McGuinness, O. Sheils, B. Sheppard, J.O'. Leary, Potential role of miR-9 and miR-223 in recurrent ovarian cancer, Mol. Cancer 7 .2008 ; 35.

50. D.L. Sprague, B.D. Elzey, S.A. Crist, T.J. Waldschmidt, R.J. Jensen, T.L. Ratliff, Platelet-mediated modulation of adaptive immunity: unique delivery of CD154 signal by platelet-derived membrane vesicles, Blood 111 .2008; 5028–5036.

51. Y. Liu, P. Jiang, K. Capkova, D. Xue, L. Ye, S.C. Sinha, N. Mackman, K.D. Janda,C. Liu, Tissue factor-activated coagulation cascade in the tumor microenvironment is critical for tumor progression and an effective target for ;

52.W. Ruf, N. Yokota, F. Schaffner, Tissue factor in cancer progression and angiogenesis,Thromb. Res. 125 .2010; S36–S38.

53.H.-M. Ko, H.H. Jung, K.H. Seo, Y.-R. Kang, H.-A. Kim, S.J. Park, H.-K. Lee, S.-Y. Im,Platelet-activating factor-induced NF-kappaB activation enhances VEGF expressionthrough a decrease in p53 activity, FEBS Lett. 580 .2006 ; 3006–3012.

54.L. Sun, Z. He, J. Ke, S. Li, X. Wu, L. Lian, X. He, X. He, J. Hu, Y. Zou, X. Wu, P. Lan,PAF receptor antagonist Ginkgolide B inhibits tumourigenesis and angiogenesis in colitis-associated cancer, Int. J. Clin. Exp. Pathol. 8.2015; 432–440.

55. B. Bussolati, L. Biancone, P. Cassoni, S. Russo, M. Rola-Pleszczynski,G. Montrucchio, G. Camussi, PAF produced by human breast cancer cells promotes migration and proliferation of tumor cells and neo-angiogenesis, Am. J. Pathol. 157 .2000 ; 1713–1725.

56.F. Bussolino, M. Arese, G. Montrucchio, L. Barra, L. Primo, R. Benelli, F. Sanavio,M. Aglietta, D. Ghigo, M.R. Rola-Pleszczynski, Platelet activating factor produced in vitro by Kaposi's sarcoma cells induces and sustains in vivo angiogenesis, J.Clin. Invest. 96 .1995 ; 940–952.

57.E.G. Robert, J.D. Hunt, Lipid messengers as targets for antiangiogenic therapy,Curr. Pharm. Des. 7 .2001 ; 1615–1626.

58.J.S. Palumbo, K.E. Talmage, J.V. Massari, C.M.L. Jeunesse, M.J. Flick,K.W. Kombrinck, M. Jirousková, J.L. Degen, Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells, Blood 105 .2005; 178–185.

59.H.-G. Kopp, T. Placke, H.R. Salih, Platelet-derived transforming growth factor-beta down-regulates NKG2D thereby inhibiting natural killer cell antitumor reactivity,Cancer Res. 69.2009; 7775–7783.

60.K. Egan, N. Cooke, D. Kenny, Living in shear: platelets protect cancer cells from shear induced damage, Clin. Exp. Metastasis 31 .2014; 697–704.

61.S. Karpatkin, E. Pearlstein, C. Ambrogio, B.S. Coller, Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo, J. Clin. Invest. 81 1988; 1012–1019.

62.O. Engebraaten, M. Trikha, S. Juell, S. Garman-Vik,. Fodstad, Inhibition of in vivo tumour growth by the blocking of host alpha(v)beta3 and alphaII(b)beta3 integrins, Anticancer Res. 29. 2009; 131–137.

63.M.R. Weber, M. Zuka, M. Lorger, M. Tschan, B.E. Torbett, A. Zijlstra, J.P. Quigley,K. Staflin, B.P. Eliceiri, J.S. Krueger, P. Marchese, Z.M. Ruggeri, B.H. Felding,Activated tumor cell integrin αv β3 cooperates with platelets to promote extravasation and metastasis from the blood stream, Thromb. Res. 140 (Suppl. 1) .2016 ; S27–36.

64.B. Felding-Habermann, T.E. O'Toole, J.W. Smith, E. Fransvea, Z.M. Ruggeri,M.H. Ginsberg, P.E. Hughes, N. Pampori, S.J. Shattil, A. Saven, B.M. Mueller,Integrin activation controls metastasis in human breast cancer, Proc. Natl. Acad.Sci. U. S. A. 98 .2001; 1853–1858.

65. E. Mammadova-Bach, P. Zigrino, C. Brucker, C. Bourdon, M. Freund, A. De Arcangelis, S.I. Abrams, G. Orend, C. Gachet, P.H. Mangin, Platelet integrin α6 β1controls lung metastasis through direct binding to cancer cell-derived ADAM9, JCI Insight 1.2016; e88245.

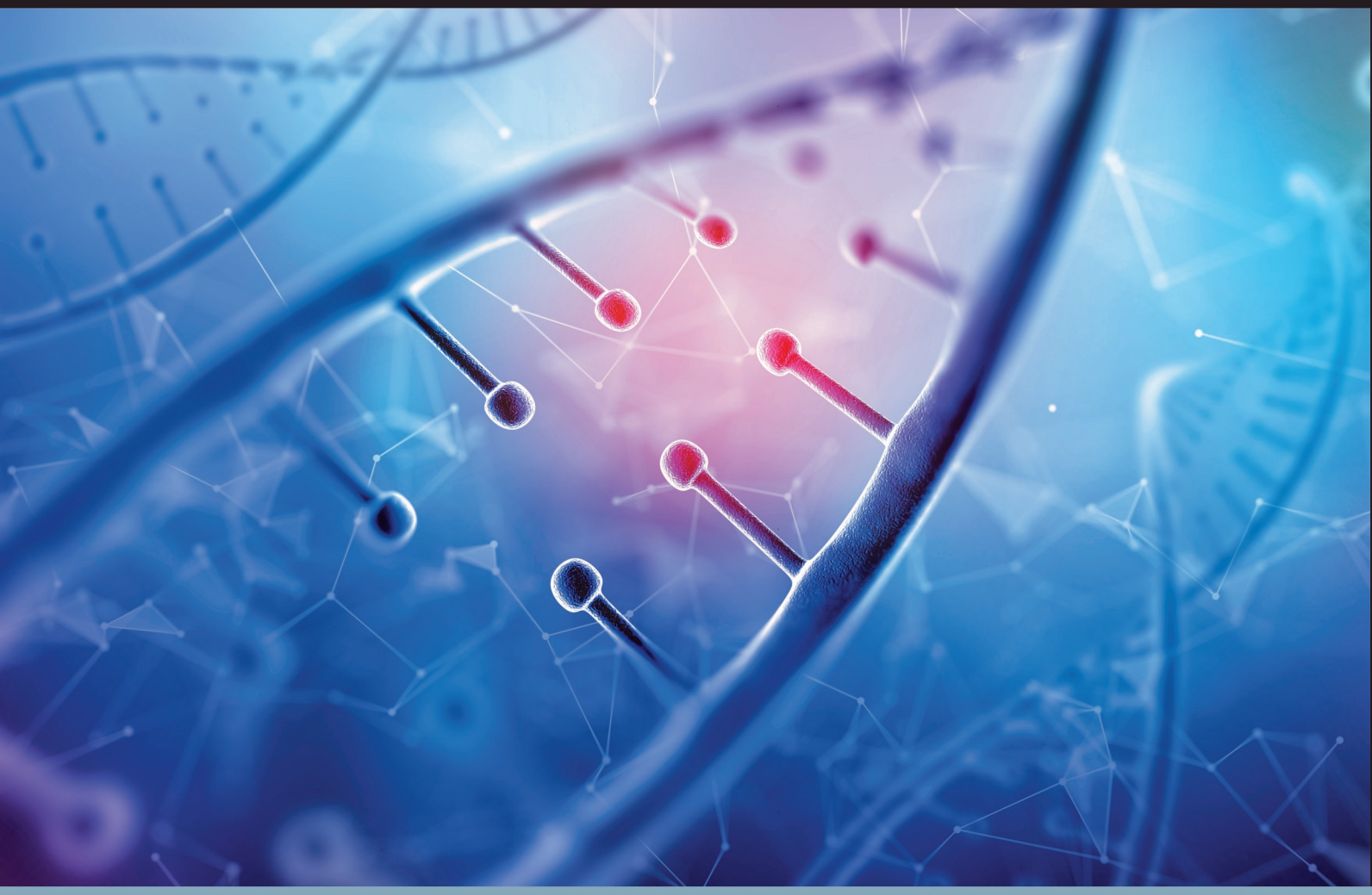
66.Y.J. Kim, L. Borsig, N.M. Varki, A. Varki, P-selectin deficiency attenuates tumor growth and metastasis, Proc. Natl. Acad. Sci. 95 .1998; 9325–9330.

67.C. Qi, B. Wei, W. Zhou, Y. Yang, B. Li, S. Guo, J. Li, J. Ye, J. Li, Q. Zhang, T. Lan,X. He, L. Cao, J. Zhou, J. Geng, L. Wang, C. Qi, B. Wei, W. Zhou, Y. Yang, B. Li,S. Guo, J. Li, J. Ye, J. Li, Q. Zhang, T. Lan, X. He, L. Cao, J. Zhou, J. Geng, L. Wang, P-selectin-mediated platelet adhesion promotes tumor growth, Oncotarget 6 .2015; 6584–6596.

.

نشریه پزشکی شخصی

فصلنامه پزشکی/ سال هشتم/ شماره بیست و نهم/ قیمت: ۱۵۰۰۰۰ ریال/ بهار ۱۴۰۲/ شماره شاپا ۳۸۶۰-۲۷۱۷



آینده علم پزشکی، شخصی محور است