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Analytical Performance Specifications (APS): whither quality?

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Abstract

It is important to establish analytical quality goals in laboratory medicine to ensure accurate and precise assessment of assay quality. Analytical performance specifications (APS) are crucial for maintaining high standards in laboratory medicine and ensuring that test results are clinically relevant and reliable for medical decision- making, as required by the ISO15189 accreditation standard for medical laboratories. By monitoring the laboratory's performance against the established APS, issues can beidentified, and corrective actions can be implemented to maintain the desired level of quality. The debate and definition of quality goals, requirements, and specifications have been ongoing since the Aspen, Stockholm, Milan, and Prague conferences. The choice of model for application in the laboratory depends on the nature of the measurement and the intended clinical application of the test. It is important to consider the level of analytical performance to which an APS is applied. However, there is a significant amount of variation in the application of clinical requirements and APS for laboratory tests, with a concerning number of laboratories not routinely evaluating methods against performance standards. The main issue is how to establishreliable clinical performance goals and determine acceptable clinical performance for laboratory tests. Currently, the statistical approaches and proposals mentioned in information exchanges are not particularly helpful. The key question is which Milan model is best to use, as all three models are naturally applied during risk assessment and considering the impact of test results on patient management/outcomes. There is a need for a future consensus conference on quality goals, specifications, or requirements.

Keywords: *Quality Control, Accreditation, Performance Specification, Method Validation*

Introduction

When setting Quality Control (QC) goals, laboratories define targets to ensure the accuracy and reliability of their test results. Westgard suggests transitioning from strict specifications to more aspirational goals, where consistently meeting a target can be considered an Acceptable Performance Specification. Goals are targets to strive for but may not always be achieved, while specifications and requirements mustbe met.[1]

Analytical performance specifications (APS) are guidelines used to assess the quality of laboratory testing processes, ensuring accuracy, reliability, and consistency over time. APS cover precision, accuracy, trueness, linearity, detection limits, and consistency across instruments and time. Key parameters in APS should include imprecision, bias, total error limits, and uncertainty of measurement, which are critical for ensuring reliable and accurate test results for clinical use.[2]

The Total Allowable Error (TEa) is the maximum error that a laboratory can allow. The Association for Diagnostics and Laboratory Medicine (ADLM), formerly AACC,has published an article explaining TEa and how much error laboratories can permit. You can find the article at the following URL:

https://www.aacc.org/cln/articles/2021/december/total-allowable-error-tea-how-much- error-can-your-laboratory-allow

(Accessed 21 June 2024).

Alternatively, one can download the Defined Acceptability Criteria or the database ofthe European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) biological variations for medical laboratory testing from the EFLM official website. The website provides a comprehensive list of biological variation estimates for various measurands, along with analytical performance specifications and reference change values. The website also offers meta-analysis-derived BV estimates for over100 measurands.

https://biologicalvariation.eu/

(Accessed 21 June 2024).

Please note that the data on the website is copyrighted by EFLM, and you maynot distribute or commercially exploit the content without their express written permission.

The importance of establishing APS for applications in clinical laboratories

It is important to establish APS for measurement procedures in clinical laboratories. The ISO standard for clinical laboratories, ISO 15189:2022, requires laboratories to validate or verify the performance of a measurement procedure for its "intended use." However, APS established for a particular scheme may not apply if a test is used for adifferent purpose than what was envisioned by the external quality assessment (EQA) provider. For instance, if a laboratory uses a glucose test to differentiate between hypoglycemic and hyperglycemic comatose patients in the Accident and Emergency (A&E) department or intensive care unit, a wider APS might be applicable than for other applications such as the diagnosis of diabetes. As EQA organizers cannot have APS for every possible intended use of a test, laboratories are advised to document their own required response to results if their use of the assay differs from the generally expected use.

ISO 15189-2022 says:

7.3.7.2 Internal quality control (IQC)[3]

a) *The laboratory shall have an IQC procedure for monitoring the ongoing validity of examination results, according to specified criteria, that verifies the attainment of theintended quality and ensures validity pertinent to clinical decision-making.*

1) *The intended clinical application of the examination should be considered, as the performance specifications for the same measurand can differ in different clinical settings.*

The performance specifications for the same measurand can differ in different clinicalsettings, and the intended clinical application of the examination should be considered. The European Federation of Clinical Chemistry and Laboratory Medicine(EFLM) has published a consensus statement on defining analytical performance specifications, which provides a framework for setting analytical performance specifications based on the intended clinical application of the examination. Probablyit is globally accepted that analytical goals should best be calculated. It was all summarized in the IUPAC/IFCC "Stockholm Conference " in 1999.[4]

The consensus reached at the 2015 Milan and 2023 Prague conferences established the ladder of possible and acceptable procedures for setting analytical goals, focusing on practical applications of performance specifications. It was emphasized that the Prague Consensus would not replace the Milan Consensus, and the latter still serves as the accepted model for performance specifications. The Prague conference aimed to demonstrate practical applications of performance specifications using three different models:

Model 1: Based on the impact of analytical performance on clinical outcomes.

Model 2: Based on components of biological variation of the measurand.

Model 3: Based on state-of-the-art analytical performance.

Furthermore, the statement suggests that the performance specifications for pre- and post-analytical laboratory processes should follow the same models as for analytical performance specifications. The principle has been widely accepted for a long time, and various tables are available to support it. The choice of model for application in the laboratory depends on the nature of the measurand and the intended clinical application of the examination. The EFLM consensus statement provides a detailed description of each model and the measurands for which they are best suited. You may find it helpful to consult the statement to determine which model is most appropriate for specific clinical applications.

External Quality Assurance (EQA) is a required laboratory activity under ISO 15189and for numerical pathology results a quantitative assessment is made comparing a laboratory's result against a target. To determine if your lab meets the APS requirements, you can participate in EQA programs. EQA organizers provide APS that indicate whether the deviation from the target value achieved by the laboratory isacceptable.

The laboratory can download the Data Analysis and Assessment Criteria Handbook from the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) website. The handbook contains information on Analytical Performance Specifications (APS), which are quality standards that participating laboratories can use to assess their performance in all RCPAQAP disciplines and respond accordingly.

For Clinical Chemistry
RCPA_302340_APS-Chemical-Pathology-Update-Dec-2022_FA.pdf(rcpaqap.com.au)

For Hematology

RCPA_302678_APS-Haematology-Document-Update_WEB.pdf (rcpaqap.com.au)

(Accessed 21 June 2024).

The handbook provides the following definitions for the terms mentioned:

- Optimal: The analytical performance specification (APS) that is desirable for a laboratory to achieve. It is defined as 0.125 $(CVi^2 + CVg^2)^{1/2}$ + 2.33 x 0.5 CVi for total error (TE) and 0.25 $(CVi^2 + CVg^2)^{1/2}$ + 2.33 x 0.25 CVi for imprecision.
- Desirable: The APS that is achievable by most laboratories. It is defined as 0.25 $(CVi^2 + CVg^2)^{1/2}$ + 2.33 x 0.5 CVi for TE and 0.5 $(CVi^2 + CVg^2)^{1/2}$ + 2.33 x 0.25CVi for imprecision.
- Minimal: The APS that is the minimum acceptable level of performance. It is defined as 0.375 $(CVi^2 + CVg^2)^{1/2}$ + 2.33 x 0.75 CVi for TE and 0.75 $(CVi^2 + CVg^2)^{1/2}$ + 2.33 x 0.25 CVi for imprecision.

The clinical and scientific/technical relevance of setting Analytical PerformanceSpecifications (APS)

1. Clinical Relevance:
   - APS ensure that laboratory results are suitable for clinical needs and can help prevent misdiagnosis and inappropriate treatment.
   - Clearly defined APS support evidence-based medicine and informed clinical decision-making.
2. Analytical Performance Validation:
   - APS provide a framework for validating the accuracy of laboratory methods and ensuring that results meet predefined quality criteria.
   - This process helps to identify and address potential sources of error, maintaining the reliability of test results.
3. Quality Assurance and Continuous Improvement:
   - APS serve as a benchmark for ongoing quality assurance and continuous improvement efforts within the laboratory.
   - Monitoring performance against APS helps identify issues and implement corrective actions.
4. Harmonization and Standardization:
   - APS contribute to the harmonization and standardization of laboratory practices across different institutions and healthcare systems, ensuring comparable and interchangeable test results.
5. Patient Safety and Confidence:
   - Adherence to well-defined APS helps to ensure patient safety and improve patient satisfaction and trust in the healthcare system.

In summary, setting Analytical Performance Specifications helps verify the quality oflaboratory results, ensure consistent validity, and support informed medical decision- making for improved patient outcomes and public health.[5]

Examples of how Analytical Performance Specifications (APS) have been successfully implemented to enhance patient care and clinical decision-making

Analytical Performance Specifications (APS) play a critical role in enhancing patient care and clinical decision-making by ensuring the reliability and clinical utility of laboratory test results, particularly for cholesterol and hemoglobin A1c (HbA1c).

For Cholesterol (Table 1):

The APS for cholesterol testing are typically defined by national and international organizations such as the Clinical and Laboratory Standards Institute (CLSI) and the National Cholesterol Education Program (NCEP).[7]

Key APS for cholesterol include:

1. Imprecision (CV): The desirable total imprecision (coefficient of variation) should be less than 3% for total cholesterol.
2. Bias: The desirable bias for total cholesterol should be less than ±3%.
3. Total Error: The total allowable error for total cholesterol should be less than ±8.9%.

These APS ensure that cholesterol test results are accurate and consistent, enabling healthcare providers to make reliable clinical decisions regarding the management of dyslipidemia and cardiovascular disease risk.[8]

For HbA1c (Table 1):

The APS for HbA1c testing are established by organizations such as the American Diabetes Association (ADA) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Key APS for HbA1c include:

1. Imprecision (CV): The desirable total imprecision (coefficient of variation) should be less than 2%.
2. Bias: The desirable bias should be less than ±2 mmol/mol (±0.2%) from the IFCC reference method.
3. Total Error: The total allowable error should be less than ±4 mmol/mol (±0.4%)from the IFCC reference method.

These APS for HbA1c ensure that the test results accurately reflect the patient's long-term glycemic control, enabling healthcare providers to make informed decisions about diabetes management, including medication adjustments, dietary changes, and lifestyle modifications.

The National Academy of Clinical Biochemistry (NACB) recommends that HbA1c assays used for diagnosis should achieve an imprecision of <3% CV and should be NGSP certified. Currently, the NGSP requires 92.5% of results to be within ±6% of a reference method traceable to the Diabetes Control and Complications Trial (DCCT).[9]

Adherence to these well-defined APS for cholesterol and HbA1c testing is crucial forproviding reliable and clinically relevant laboratory data that supports healthcare providers in making appropriate clinical decisions and improving patient outcomes.

Discussion

The original Milan consensus papers were in a special issue of CCLM. The following paper was the executive summary of multiple articles published in the same issue: Clin Chem Lab Med 2015; 53(6): 833–835.

Free to download at: https://www.eflm.eu/files/efcc/3.5%20CCLM-Consensus%20Statement.pdf

(Accessed 21 June 2024)

The experts in Aspen, Stockholm, Milan, and Prague have been refining the definitions of quality goals, requirements, and specifications. The recent meeting in Prague did not result in a consensus, and different proposals were put forward. These include publishing a list of approximately 100 analytes with performance specifications, diagnostic manufacturers providing more information on the uncertainty of their reference assignments, standardizing performance specifications,expanding the database to include state-of-the-art and clinical outcome goals, and having multiple performance specifications for the same analyte.

Chicken and egg situation

When considering clinical guidelines for medical needs, it can feel like a chicken andegg situation - which came first? In my opinion, most clinical guidelines are based on research and analysis of laboratory data. For instance, cutoff values of cholesterol and HbA1c for cardiovascular risk assessment and the diagnosis of diabetes mellitus are established based on studies like the Framingham Heart Study[10] and the ADA recommendations[11], respectively. Medical doctors usually trust that the laboratory hasselected the right methodology and do not question the reliability of the results.

However, laboratory directors, usually pathologists in most countries, should be responsible for setting the analytical goals and specifications for their laboratory teststhat impact medical decisions and estimating the measurement uncertainty. This requires a collaborative effort involving laboratory management, quality experts, technical personnel, and most importantly, the latest technology available on the market. It is essential to consider the level of analytical performance to which an analytical performance specification (APS) is applied. The state-of-the-art defines the highest technically achievable analytical performance at the time it is measured, as demonstrated by the participating laboratories in the EQA program.

Specifications/requirements have to be met

A goal is something you strive to achieve but may not always reach. Anything that you cannot achieve becomes a goal. The Prague Conference on Analytical Performance Specifications has been an important platform for discussing quality standards in laboratory medicine. In the most recent symposium held in October 2023, there was a significant change in terminology. Instead of using the term "analytical performance specifications" (APS), the conference proposed the use of "analytical performance goals" (APG) for performance targets that may not currently be achievable due to technological limitations. Essentially, if a specification cannot be met, it becomes a goal. However, specifications that can be achieved still fall under the APS category. This change reflects a practical approach to setting realistic expectations while maintaining quality standards.

The attainment of biological goals may not always be possible, unlike goals related to cholesterol and HbA1c. Clinical goals, such as those for blood gas analysis, calcium, and glucose, vary depending on the clinical environment. For example, the TEa limits for PO2 differ between an operating room and a remote hospital, and tighter limits forcalcium are used for samples from neonatal intensive care compared to general adult screening. Additionally, there are differences between glucose point-of-care (POC) and laboratory results. It is important to consider what

healthcare providers, physicians, and patients aim to achieve, whether it's screening, diagnosis, assessing the severity of a condition, or exploring treatment possibilities. This complexity will continue to provide academia, literature, and industry with numerous interesting tasks and findings for many years to come.

The draft guidance for performance goals at the Milan conference initially suggested clinicians' opinions as the most important criterion, but this idea was later removed from the final guidance. An article in the CCLM collection pointed out that, at least in Italy, there is some work to be done to promote the concept and the use of APS because many laboratorians and clinicians may not understand basic principles.[12]

It's important to recognize that assays serve different purposes, such as screening, diagnosis, and monitoring, and a single goal cannot be universally applicable to all. Furthermore, values that do not meet goals should not be considered equally problematic. Major errors can cause more significant issues than errors that only slightly exceed the goals.

The measurement of APS depends on the model used to set the specifications

The EFLM consensus statement provides detailed guidance on how to measure APS for each model. For example, Model 2 is based on the components of biological variation of the measurand. The APS for Model 2 can be measured by calculating the total allowable error (TEa), which is the maximum permissible difference between the true value of the measurand and the measured value that is consistent with the biological variation of the measurand. The TEa can be calculated using the following formula:

$$TEa = 1.65 \times CVi \times (\mu + CVg)$$

where CVi is the within-subject biological variation, μ is the analytical measurement uncertainty, and CVg is the between-subject biological variation. The EFLM consensus statement provides detailed guidance on how to calculate the TEa for different measurands.[13,14]

Tonks' Formula

It's crucial to determine which Milan model is best suited for use when conducting a risk assessment and evaluating the impact of test results on patient management and outcomes. Graham Jones has explained this in his papers in a practical and less theoretical manner. All measurements must be appropriate for their intended purpose. In laboratory medicine, the purpose of measurements is to differentiate between healthy and diseased individuals. To accomplish this, a reference range or interval for a test must be established to aid in clinical decision-making. Reference intervals are the inverse of biological variation. Therefore, the EFLM's systematic work on biological variation is important, even though the full implications are not yet fully understood.

As an idea and an initiative, instead of just considering it, why not compile a list of TEa based on Tonks' rule (formula) from an article published in 1963? Reference ranges (RR) or intervals (RI) are essential for clinical decision-making, and every laboratory should include RR/RI along with the report. Although some of the calculated TEa by Tonks' rule may be larger than necessary, it is still the cutting-edge technology that most laboratories can achieve.

You can see examples of typical results in Table 1 of the original 1963 article. https://www.qualitat.cc/sitebuildercontent/sitebuilderfiles/tonks.1963.pdf (Accessed 21 June 2024)

Disadvantages: The reference intervals may be too wide, laboratory-defined, often revised, or not established appropriately. They may not apply to

cutoff values.

It's evident that our world is far from perfect, and therefore, selecting Milan or any other criteria for the APS option may not be suitable for all situations in the field of laboratory medicine. Let's work together to focus on larger systems rather than getting caught up in details (zero-sum mindset). We may need to incorporate additional tools and perspectives to achieve this.

There are a lot of papers on APS in CCLM. This is a hotly debated topic in Europe. See the link for a collection:
https://www.degruyter.com/search?query=analytical+performance+specifications&startItem=0&pageSize=10&sortBy=relevance&documentVisibility=all

(Accessed 21 June 2024)

Performance specifications are crucial in maintaining high standards in laboratory medicine, ensuring that test results are clinically relevant and trustworthy for medical decision-making. The selection of the model for use in the laboratory depends on the nature of the measurement and the intended clinical application of the test. However, even after 25 years (since 1999) of recognizing the importance of clinical requirements and APS for laboratory tests, there is still a wide variety of applications and a concerning number of laboratories that do not regularly evaluate methods against performance standards. The mindset of "If the QC chart looks good, everything is fine. Just expand the QC chart range to make it look good" raises the question of why doing the wrong thing often feels like the right thing to do. In short, maintaining a specific standard of performance for a given test is likely more important than setting the performance standard. Minimizing spurious gross errors is the first priority in providing reliable tools for clinicians, ensuring they have trustworthy information to guide patient care. The primary issue still remains – how to establish reliable clinical performance goals and what constitutes acceptable clinical performance for laboratory tests. Performance specifications for the same analyte could vary based on different clinical uses and geographical locations. The key question is which Milan model is best to use, as all three models are naturally utilized in risk assessment to consider the impact of test results on patient management and outcomes.[15]

Conclusion

In conclusion, it is essential to establish appropriate Analytical Performance Specifications to ensure the quality of analytical results. This, in turn, supports reliable clinical decision-making, patient care, and other applications. Achieving this requires a collaborative, evidence-based approach that balances the needs of stakeholders with rigorous analytical performance requirements. However, the statistical approaches and proposals mentioned in the information exchange, such as choosing minimal, desirable, or optimal acceptable performance limits, are not particularly helpful. The total error of 95% of results doesn't work because 5% of results are unspecified. It is necessary to have a future consensus conference on quality goals, specifications, or requirements. By addressing these challenges, laboratories can establish effective protocols to maintain the desired level of quality and reliability in their analytical results. Stay tuned.

References

1. Westgard S. So long, Specifications! Gear up for Goals. Westgard QC 7614 GrayFox Trail, Madison, Wisconsin 53717, February 2024. https://westgard.com/essays/quality-requirements-and-standards/1057-so-long-specifications.html (Accessed 21 June 2024)

2. Dalenberg DA, Schryver PG, Klee GG. Analytical performance specifications: relating laboratory performance to quality required for intended clinical use. ClinLab Med 2013;33:55-73.

3. International Organization for Standardization (ISO). ISO/IEC 15189:2022 Medical laboratories requirements for quality and competence. Geneva (Switzerland), December 2022.

4. HKAML. A guide on how to implement internal quality control (IQC). Edited by Richard Pang. Published by Hong Kong Association of Medical Laboratories (HKAML), March 2024. http://www.hkaml.org/images/Documents/QR%20Code%20Version%202024%20HKAML%20A%20How-to%20Guide%20to%20IQC-V02.pdf (Accessed 21 June2024)

5. Fraser CG. The 1999 Stockholm Consensus Conference on quality specifications in laboratory medicine. Clin Chem Lab Med 2015;53:837-40. https://doi.org/10.1515/cclm-2014-0914

6. Jones GRD. Using analytical performance specifications in a medical laboratory.From the Journal Clinical Chemistry and Laboratory Medicine (CCLM) https://doi.org/10.1515/cclm-2024-0102 Published online by De Gruyter April 16, 2024.

7. CDC Clinical Standardization Programs. The CDC Cholesterol Reference Method Laboratory Network (CRMLN) Total Cholesterol (TC) Certification Protocol Using TC-IDMS RMP (Released: July 2022) https://www.cdc.gov/clinical-standardization-programs/media/pdfs/2024/04/TC-IDMS-CRMLN-Cert-Protocol- 508.pdf (Accessed 26 June 2024).

8. Cole J, Sampson M, van Deventer HE, et al. Reducing Lipid Panel Error Allowances to Improve the Accuracy of Cardiovascular Risk Stratification. Clin Chem 2023;69:1145-54.

9. Sacks DB, Arnold M, Bakris GL, et al. Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. Diabetes Care 2023;46:e151–e199.

10. Kreger BE, Odell PM, D'Agostino RB, et al. Long-term intraindividual cholesterol variability: natural course and adverse impact on morbidity and mortality—the Framingham Study. Am Heart J. 1994;127:1607–14.

11. American Diabetes Association Professional Practice Committee. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes-2024. Diabetes Care 2024;47(Supplement_1):S20–S42 https://doi.org/10.2337/dc24-S002. https://diabetesjournals.org/care/article/47/Supplement_1/S20/153954/2- Diagnosis-

and-Classification-of-Diabetes (Accessed 21 June 2024).

12. Ceriotti F, Buoro S, Pasotti F. How clinical laboratories select and use Analytical Performance Specifications (APS) in Italy. From the Journal Clinical Chemistry and Laboratory Medicine (CCLM) https://doi.org/10.1515/cclm-2023-1314; published online February 28, 2024.

13. Jones GRD, Albarede S, Kesseler D, et al. for the EFLM Task Finish Group – Analytical Performance Specifications for EQAS (TFG-APSEQA). Clin Chem Lab Med 2017;55:949–955.

14. Sandberg S, Coskun A, Carobene A, et al. Analytical performance specifications based on biological variation data – considerations, strengths, and limitations. ClinChem Lab Med 2024: https://doi.org/10.1515/cclm-2024-0108.

15. Oosterhuis W. Gross overestimation of Total Allowable Error based on biological variation. Letter to Editor. Clin Chem 2011;57:1334-6.

Table 1. Examples of how Analytical Performance Specifications (APS) have beensuccessfully implemented to enhance patient care and clinical decision-making

APS Parameters	Cholesterol		HbA1c	
Imprecision				
• CLIA[1]	n/a	CRMLN/CDC[4]	n/a	NGSP/CAP[5]
• RCPAQAP[2]	n/a	≤ 3%	n/a	≤2.0%
• EFLM (Biological Variation)[3]	2.6%		0.6%	
Bias				
• CLIA[1]	n/a	CRMLN/CDC[4]	n/a	NGSP/CAP[5]
• RCPAQAP[2]	n/a	≤ ± 3%	n/a	≤ ± 3.0%
• EFLM (Biological Variation)[3]	± 4.0%		± 1.4%	
Total Error				
• CLIA[1]	± 10%	CRMLN/CDC[4]	± 8.0%	NGSP/CAP[5]
• RCPAQAP[2]	± 6.0%	≤ ± 8.9%	± 6.0%	≤ ± 6.0%
• EFLM (Biological Variation)[3]	± 8.3%		± 2.4%	

1. 2024 CLIA Proposed Acceptance Limits for Proficiency Testing - Westgard https://westgard.com/clia-a-quality/quality-requirements/1002-2024-clia-requirements.html
2. Chemical Pathology Analytical Performance Specifications - RCPAQAP https://rcpaqap.com.au/resources/chemical-pathology-analytical-performance-specifications/
3. EFLM Biological Variation Database https://biologicalvariation.eu. (Desirable Limits)
4. The Cholesterol Reference Method Laboratory Network (CRMLN), Centers for Disease Control and Prevention (CDC) https://www.cdc.gov/clinical-standardization-programs/php/cvd/improving-performance-crmln.html
5. National Glycohemoglobin Standardization Program (NGSP), College of American Pathologists (CAP) Survey Data https://ngsp.org/CAPdata.asp

n/a: Not available

Citrin Deficiency, is it an uncommon disease? *A mini topic update*.

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Introduction

Citrin is a protein expressed in the liver, kidney, heart, and small intestine. Citrin involves several pathways, including the malate-aspartate shuttle and the malate-citrate shuttle. It transforms aspartate from mitochondria to cytosol in exchange for glutamate, making aspartate available for ligation to citrulline in the next step of the urea cycle. Citrin also transfers cytosolic nicotinamide adenine dinucleotide hydrogen (NADH)-reducing equivalents into mitochondria, an essential shuttle for hepatic glycolysis and coupled lipogenesis.

Deficiency or dysfunction of this transporter, a hereditary disorder caused by SLC25A13 mutations, affects the urea cycle, energy production in hepatocytes and results in impaired glucose utilization and galactose metabolism. A dysfunctional NADH shuttle increases the cytosolic NADH level and decreases the mitochondrial NADH level. The elevation of cytosolic NADH/NAD+ impairs glucose utilisation in glycolysis. A reduced mitochondrial NADH/NAD+ impairs the beta-oxidation pathway, resulting in an energy deficit in hepatocytes and inadequate ketone production during hypoglycemia events. Moreover, deficiency or dysfunction of this transporter results in decreased aspartate in the cytoplasm, which limits argininosuccinate synthase 1 (ASS1) enzyme activity. This results in increased plasma citrulline concentrations, as observed in citrullinemia Type I, mostly in neonatal presentation. An impaired urea cycle leads to hyperammonemia.

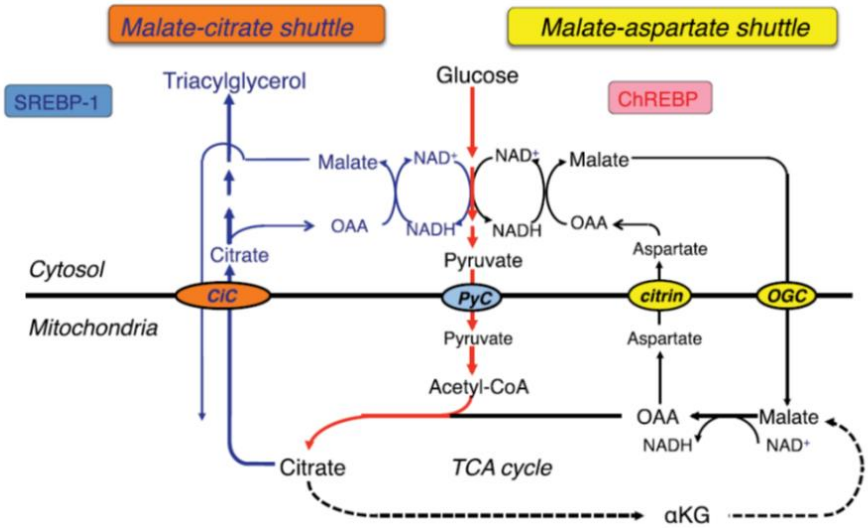

Figure 1. The role of Citrin in the malate-aspartate shuttle and malate-citrate shuttle Glycolysis and lipogenesis in the liver. Hayasaka K, Numakura C. Adult-onset type II citrullinemia: Current insights and therapy. Appl Clin Genet. 2018 Dec 12;11:163-170.

Citrin deficiency was initially described in the Japanese population and was considered an East Asian condition. In recent years, however, patients in North America and Europe have been identified, and the condition is now widely viewed as a pan-ethnic disease. Citrin deficiency (CD) is an autosomal recessive disease caused by the SLC25A13 gene mutation. It includes three age-dependent clinical phenotypes (figure 2): neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), adult-onset type II citrullinemia (CTLN2) and failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD) between NICCD and CTLN2 stages. Although NICCD is a pan-ethnic disease we recognized, it is still prevalent in East Asia. Meanwhile, China is a high-incidence area, and the mutation carrier rate of the SLC25A13 gene is as high as 1/65. The type of variation of SLC25A13 is also different in different regions. A total of >11,000 individuals are estimated to be homozygous or compound heterozygous for SLC25A13 pathogenic variants in the Guangdong province in southern China. Currently, >80 SLC25A13 pathogenic variants have been identified, but among them, four prevalent mutations c.851_854del (p.R284fs286X), c.1638_1660dup (p.A554fs570X), IVS6+5G>A (p.A206fs212X), and IVS16ins3kb (p.A584fs585X) account for >80% of the Chinese population who exhibit citrin deficiency. In citrin deficiency, impaired energy production in hepatocytes and less triacylglycerol would be available via hepatic lipogenesis for the cholesterol metabolism, which plays a significant role in fat supply during growth spurt periods. As such, the clinical consequence of citrin deficiency is growth impairment, impaired liver function, dyslipidemia, and fatty liver. Most citrin deficiency patients (NICCD or CTLN2) present with cholestasis of fatty liver, though pathophysiology is not entirely understood. The underlying main reason is an energy deficit in the liver. Often, citrin deficiency is characterized by a strong preference for protein-rich and/or lipid-rich foods and aversion to carbohydrate-rich foods. In adult-onset type II citrullinemia CTLN2, the clinical presentation of these phenotypes is summarized in Figure 2.

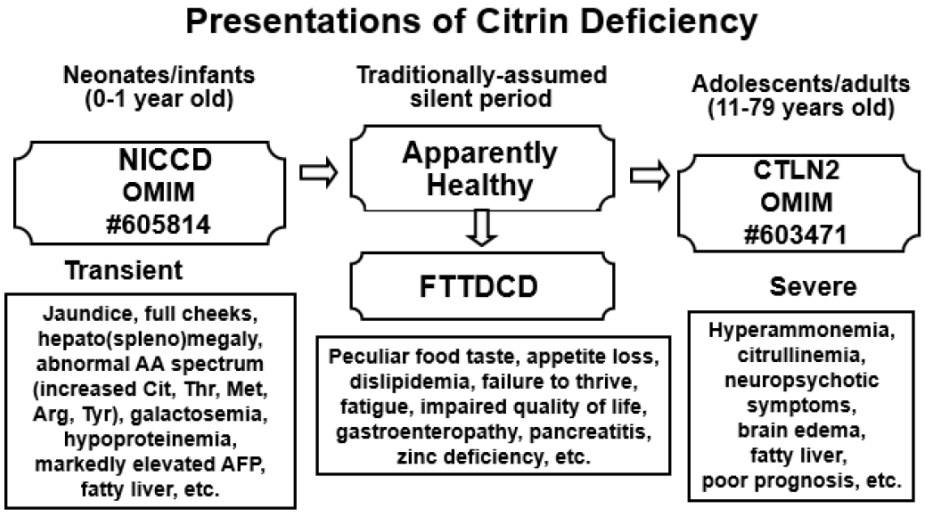

Figure 1. Clinical and laboratory manifestations of citrin deficiency

AA = amino acids; AFP = alpha-fetoprotein; Arg = arginine; Cit = citrulline; CTLN2 = adult-onset type II citrullinemia; FTTDCD = failure to thrive and dyslipidemia caused by citrin deficiency; Met = methionine; NICCD = neonatal intrahepatic cholestasis caused by citrin deficiency; Thr = threonine; Tyr = tyrosine

Figure 2. Clinical and laboratory manifestations of citrin deficiency

Saheki T, Song YZ. Citrin Deficiency. [Updated 2017 Aug 10]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews®

Diagnosis for citrin deficiency

The diagnosis of citrin deficiency is established in an individual with characteristic biochemical findings. Diagnosis is firstly based on the measurement of these critical metabolites and then on the enzyme (citrullinemia type 1 -argininosuccinate synthetase deficiency and argininosuccinic aciduria-argininosuccinate lyase deficiency) or genetic studies for differential diagnosis. The diagnosis of a metabolic disorder is always a tricky process which needs a complete synthesis between the clinical picture and the biochemical and biomarker profile. Therefore, it is essential to have a high index of suspicion to diagnose this disorder. Increased blood or plasma concentration of ammonia, plasma or serum concentration of citrulline and arginine, and plasma or serum threonine-to-serine ratio were found.

Laboratory investigations indicate signs of cholestasis and liver synthetic dysfunction, which may also indicate the possibility of citrin deficiency. Elevated liver transaminases, alkaline phosphatase, γ-glutamyltranspeptidase levels, abnormal coagulation times, protein, glucose, conjugated bilirubin, and bile acid levels were also found.

Biochemically, plasma ammonia, citrulline, and arginine levels are elevated in CLTN2, while hyperbilirubinemia, hypertyrosinemia, and hypermethioninemia seen in NICCD are absent. For confirmation, the identification of biallelic pathogenic variants in SLC25A13 by molecular studies was also performed.

The first-tier MS/MS screening method using conventional marker metabolites alone is associated with a relatively low specificity. It is susceptible to a high false-positive rate, imprecision, and false negatives since the adequate cutoff citrulline level is difficult to determine. False-negative rates are high for citrin deficiency and may delay diagnosis and treatment in patients. To circumvent unnecessary concerns for parents and newborns in false positives and to increase the specificity of newborn screening for disorders without a particular primary marker, second-tier strategies have been developed. Second-tier NGS tests could help rule out ambiguous screening results due to nutritional impacts or common heterozygous carriers while ruling out IEM cases with unsatisfactory sensitivity using MS/MS, such as citrin deficiency. Several studies of molecular second-tier testing were validated in several reports, and the detection rate was increased to a rate similar to the expected rate estimated.

Management of citrin deficiency patients

Given the multiple metabolic pathways involved in citrin deficiency and its diversities in various phenotypes, management of citrin-deficient patients: Low protein and high CHO diet will worsen, and infusion of glucose, fructose, and glycerol is contraindicated. Alcohol may trigger CTLN2, while the medication prescribed needs close monitoring.

The current treatment strategy will be mainly on diet therapy, with low carbohydrate, high protein and high fat, given impaired glucose utilization and also initiated the use of lactose-free, medium chain triglyceride MCT enriched formula, which facilitates direct absorption from the intestine without bile fluid and also direct energy to the hepatic cells by acetyl CoA, through TCA cycle—other treatments including administration of sodium pyruvate in addition to dietary control.

Treatment for cholestasis has been shown to delay the onset of Adult-onset type II citrullinemia CTLN2 and improve prognosis. In case of impaired liver function, liver supportive therapy, including fat-soluble vitamins, FFP, albumin ammonia scavengers and in more severe cases, a liver transplant might be considered, which prevents episodic hyperammonemia crises and corrects the metabolic disturbances. Because intense treatment,

such as liver transplantation, may become necessary to cure CTLN2, effective preventative treatment during the adaptation/compensation stage is very important.

Summary

Citrullinemia type 2 is expected in East Asians (including Hong Kong) and usually presents in adults with hyperammonemia and neuropsychiatric disease (CTLN2). Citrin deficiency presentation can be highly variable. It may also cause neonatal/infantile cholestatic liver disease without hyperammonemia (NICCD), which is primarily transient, and failure to thrive and dyslipidemia with hypoglycemic attacks (FTTDCD) mainly after one year of age.

Tandem mass spectrometry (MS/MS) program for IEM for early detection and diagnosis in newborn screening is widely adopted. When dried blood spot(DBS) or plasma amino acid analysis was performed, elevated levels of citrulline, methionine, tyrosine, phenylalanine, threonine, lysine, etc, may be observed. This allowed rapid, cost-effective, and simultaneous detection of analytes related to IEM, yet there still needs to be some limitations due to the high false negative rate. It is essential to investigate the diagnostic possibility of citrin deficiency even in patients with average newborn screening results.

Application of second-tier strategies in NBS allows for increased specificity and, consecutively, a higher PPV of NBS. If clinically indicated, the family cascade screening of carrier status for at-risk relatives should be carried out since it can be asymptomatic in the early stages. One potential challenge encountered in testing genetic variants is the management of these carriers. Since the primary purpose of the newborn screening program is to detect patients and improve their prognoses, reporting other information, such as carrier status, is not intended to be a part of this program during second-tiered genetic testing.

References

1. Hayasaka K, Numakura C. Adult-onset type II citrullinemia: Current insights and therapy. Appl Clin Genet. 2018 Dec 12;11:163-170

2. Saheki T, Song YZ. Citrin Deficiency. 2005 Sep 16 [Updated 2017 Aug 10]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews®

3. Therrell, BL, et al.. Current status of newborn screening worldwide: 2015. Semin Perinatol 2015;39:171–87.

4. Chong SC, Lo P, Chow CW, Yuen L, Chu WCW, Leung TY, Hui J, Scaglia F. Molecular and clinical characterization of citrin deficiency in a cohort of Chinese patients in Hong Kong. Mol Genet Metab Rep. 2018 Sep 1;17:3-8.

5. Lehmann, S, Delaby, C, Vialaret, J, Ducos, J, Hirtz, C. Current and future use of “dried blood spot” analyses in clinical chemistry. Clin Chem Lab Med 2013;51:1897–909

6. Wang, LY, Chen, NI, Chen, PW, et al. Newborn screening for citrin deficiency and carnitine uptake defect using second-tier molecular tests. BMC Med Genet 2013;14:24.

7. Chen CY, Chang MH, Chen HL, Chien YH, Wu JF. The prognosis of citrin deficiency differs between early-identified newborn and later-onset symptomatic infants. Pediatr Res. 2023 Sep;94(3):1151-1157

8. Hayasaka K, Numakura C, Toyota K, et al. Medium-chain triglyceride supplementation under a low-carbohydrate formula is a promising therapy for

adult-onset type II citrullinemia. Mol Genet Metab Rep. 2014 Jan 14;1:42-50.

9. Wang K, Zou B, Chen F, Zhang J, Huang Z and Shu S Case report: Three novel variants on SLC25A13 in four infants with neonatal intrahepatic cholestasis caused by citrin deficiency. Front. Pediatr. 2023 11:1103877.

10. The Task Force on the Pilot Study of Newborn Screening for Inborn Errors of Metabolism, Hong Kong SAR. Evaluation of the 18-month “Pilot Study of Newborn Screening for Inborn Errors of Metabolism” in Hong Kong. HK J Paediatr (New Ser) 2020;25:16-22.

11. Mak, Chloe, Papassotiriou, Ioannis, Zierk, Jakob, Kohse, Klaus P., Greaves, Ronda F., Geaghan, Sharon M. D., Lang, Tim, Loh, Tze Ping and for the IFCC Committee on Emerging Technologies in Pediatric Laboratory Medicine,. "Emerging technologies in paediatric laboratory medicine" Journal of Laboratory Medicine, vol. 45, no. 6, 2021, pp. 245-248.

12. Kwok Yeung Tsang, Toby Chun Hei Chan, Matthew Chun Wing Yeung, Tsz Ki Wong, Wan Ting Lau and Chloe Miu Mak. Validation of amplicon-based next generation sequencing panel for second-tier test in newborn screening for inborn errors of metabolism. J Lab Med 2021; 45(6): 267–274

13. Hui-An Chen et al.Improved diagnosis of citrin deficiency by newborn screening using a molecular second-tier test. Molecular Genetics and Metabolism Volume 136, Issue 4, August 2022, Pages 330-336

14. Yin-Hsiu Chien, Wuh-Liang Hwu. Mini Review Article The modern face of newborn screening. Pediatrics and Neonatology 64 2023 S22 -291

15. Qinlong Zeng et al. Rapid Genetic Diagnosis of Citrin Deficiency by Multicolor Melting Curve Analysis.

16. Front. Pediatr., 05 May 2021 Sec. Genetics of Common and Rare Diseases Volume 9 - 2021

17. Gramer, Gwendolyn and Hoffmann, Georg F.. "Second-tier strategies in newborn screening – potential and limitations" Medizinische Genetik, vol. 34, no. 1, 2022, pp. 21-28.

18. Kido J, Häberle J, Tanaka et al. Improved sensitivity and specificity for citrin deficiency using selected amino acids and acylcarnitines in the newborn screening. J Inherit Metab Dis. 2023 Sep 8.

19. Hayasaka K, Numakura C, Toyota K, et al. Medium-chain triglyceride supplementation under a low-carbohydrate formula is a promising therapy for adult-onset type II citrullinemia. Mol Genet Metab Rep. 2014;1:42–50.

20. Hayasaka K, Numakura C, Toyota K, Kimura T. Treatment with lactose (galactose)-restricted and medium-chain triglyceride-supplemented formula for neonatal intrahepatic cholestasis caused by citrin deficiency. JIMD Rep. 2012;2:37–44.

21. Hirai I, Kimura W, Suto K, et al. Living donor liver transplantation for type II citrullinemia from a heterozygous donor. Hepatogastroenterology. 2008;55:2211–6.

22. Bodian DL, Klein E, Iyer RK, et al. Utility of whole-genome sequencing for detection of newborn screening disorders in a population cohort of 1,696 neonates. Genet Med. 2016 Mar;18(3):221-30.

A retrospective study of varicella immunisation in Hong Kong

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Abstract

In Hong Kong, the high prevalence of varicella places a considerable burden on the public healthcare system. A universal varicella vaccination programme was started in July 2014 to reduce the varicella infection rate. Since then, there have been limited studies assessing the changes in varicella immunisation and epidemiology in the Hong Kong population after implementing the programme. We conducted a retrospective study to assess the varicella-zoster virus (VZV) immunoglobin G (IgG) seroprevalence between 2015 and 2020 by extracting VZV IgG serological data of 3150 cases from the laboratory information system of a local private medical laboratory. We also reviewed the varicella notifications from 1999 to 2020 and the varicella serological data in 2000, 2005, 2010 and 2015 listed on the Centre for Health Protection (CHP) website. The overall seroprevalence estimate was 96.4% (95% CI: 95.8% – 97.1%). There was no significant difference in VZV IgG seroprevalence in the adult population between 2015 and 2020, X^2 (5, N = 3088) = 8.06, p > .05. Gender differences were identified, with a higher VZV IgG seroprevalence rate in the female population, X^2 (1, N = 3088) = 4.37, p = .037. The data of age-specific VZV IgG seroprevalence rates are consistent with the data from the CHP, with >70% of individuals acquiring immunity against VZV before reaching adulthood. A dramatic increase in the VZV IgG seroprevalence rate in children aged 1 – 4 and a gradual decline in the annual rate of varicella notification were observed. It is concluded that a high VZV IgG seroprevalence rate was identified in the Hong Kong population, with a noticeably high prevalence of varicella among the younger population of <25 years. Implementing the universal varicella vaccination programme is essential in providing VZV immunity to and preventing varicella infections among children.

Keywords: *Varicella-zoster virus; Universal vaccination; Seroprevalence*

Introduction

A primary infection with varicella-zoster virus (VZV) in humans can result in a highly infectious and contagious disease known as varicella (chickenpox), predominantly acquired in children below 12. The mode of transmission of varicella includes airborne and /or droplet information and direct or indirect contact with the discharges from the skin lesions of infected individuals.[1] The primary VZV infection begins with the local VZV replication in the upper respiratory mucosa.[2] The spreading of VZV follows local replication of VZV to tonsillar and regional lymphoid tissues via VZV-infected Langerhans cells, where T lymphocytes become infected with VZV.[3] VZV-infected T lymphocytes are reconfigured into activated memory T lymphocytes with reduced immune activities and increased skin-homing capacity.[4] As a result, VZV is transported by the infected T lymphocytes throughout the body via the bloodstream and the lymphatic system and eventually disseminated majorly to the skin during primary infection, leading to the appearance of the characteristic vesiculopustular rash on the skin. Although the varicella infection is generally mild, benign and self-limited, varicella can cause severe complications, including secondary bacterial infections, pneumonia, encephalitis or haemorrhagic problems, especially in people with weakened immunity or women with pregnancy.[5]

In the pre-vaccine era, VZV infections occur worldwide, causing essentially universal exposure to VZV. In high-income developed countries, the incidence of varicella occurred commonly among preschool and school-aged children, with more than 90% of infections occurring by the age of 17 years; only less than 5% of adults remained susceptible to VZV without seroconversion.[6] After developing and implementing the varicella vaccination programme, varicella infections and severe complications have dramatically reduced. In the United States, the hospitalisations and deaths caused by varicella have significantly reduced by 93% and 94%, respectively, since 1996, when varicella vaccine programmes for children aged 12 to 18 months were implemented.[7] Interestingly, the incidence of varicella also declined in all age groups, including infants who are not eligible for the vaccination program and adults who have not been vaccinated, indicating the benefits of herd immunity from the vaccination programme.[8]

In Hong Kong, varicella infection has long been the most commonly notifiable infectious disease reported. From 2010 to 2019, the average annual rate of varicella notifications was 52.1% of all reported notifiable infectious diseases. Notably, the high prevalence of VZV-associated infections places a substantial economic burden on the public healthcare system and increases the burden associated with the shortage of medical and healthcare personnel in Hong Kong public hospitals. Therefore, it is crucial to minimise VZV infections, thereby reducing the burden associated with VZV infections. Vaccines against varicella have been available in Hong Kong's private market since 1996. Subsequently, the varicella vaccine was listed and scheduled in the Hong Kong Childhood Immunisation Programme in July 2014 to implement universal varicella vaccination in Hong Kong. Under the programme, eligible children born on or after 1 January 2013 should receive a two-dose scheduled varicella vaccine.[9]

Although several studies assessed the trends of varicella immunisation and the change in varicella's epidemiology between 1996 and 2014, minimal studies have been conducted in the Hong Kong population to assess these areas after implementing

the universal varicella vaccination programme. It is important to start assessing the trends of varicella immunisation to evaluate the effectiveness and importance of the universal varicella vaccination programme in changing the epidemiology of and preventing VZV transmission and its ability to reduce the disease burden associated with VZV infections. While serological data is essential to determine the effectiveness of the universal varicella vaccination programme, we conducted a retrospective study to investigate the VZV immunoglobin G (IgG) antibodies in the Hong Kong population between 2015 and 2020. This study aimed to assess the change of VZV IgG seroprevalence in Hong Kong after implementing the universal varicella vaccination. We also described the gender- and age-specific profiles of VZV seroprevalence in Hong Kong. Besides, we reviewed the varicella notifications and VZV IgG serological data from the public sector in Hong Kong.

Methodology

Study design

A retrospective study investigated the seroprevalence of VZV IgG antibodies between 2015 and 2020 in the Hong Kong Special Administrative Region.

Sera collection

The presence of immunity to VZV in the general population was determined by evaluating the serum IgG antibodies specific to VZV levels in the human blood samples. The blood samples were collected in serum separation transport tubes aseptically via venepuncture. All blood samples collected were centrifuged at 3400 rpm for 15 minutes to separate the serum from cellular elements. The separated serum is the ideal sample for determining the VZV IgG antibody levels.

Laboratory test

VZV IgG antibodies were measured in human sera by an indirect enzyme-linked immunosorbent assay (ELISA) using the Euroimmun anti-VZV IgG ELISA test kit (Euroimmun AG, Germany), which allows semi-quantitative detection of VZV IgG antibodies in the serum samples. The detection process involved adding 100 µl of the negative and positive controls, Euroimmun calibrator, and patient samples into the individual reaction wells according to the manufacturer's pipetting protocol, and the reaction wells were incubated at room temperature (18 to 25℃) for 30 minutes. After incubation, it emptied and washed the reaction wells thrice with 300 µl of wash buffer per wash. Then, pipetting 100 µl of enzyme-conjugated anti-human IgG antibodies to each reaction well and incubated at room temperature (18 to 25℃) for 30 minutes. After the conjugate incubation, emptying and washing the reaction wells as described above. Subsequently, 100 µl of substrate solution was added into each reaction well to allow substrate incubation at room temperature (18 to 25℃) for 15 minutes, and the process of substrate incubation was protected from direct sunlight. After that, the substrate incubation was stopped by pipetting 100 µl of stop solution into each reaction well. The ELISA microplate was slightly shaken before the subsequent measurement to ensure a homogeneous distribution of the solution. Finally, the light intensity in each reaction well was measured via photometric measurement at a wavelength of 450 nm within 30 minutes of the addition of the stop solution. A wavelength between 620 nm and 650 nm was selected as the reference wavelength. The testing result of VZV IgG antibodies was expressed in the ratio of the extinction value in a patient sample over the extinction value in the Euroimmun calibrator

containing 100 international units per litre (IU/L) of VZV IgG antibodies. Calculating the ratio according to the following formula given by the manufacturer's instructions:

$$\frac{Extinction\ value\ of\ the\ patient\ sample}{Extinction\ value\ of\ calibrator} = Ratio$$

The results were interpreted according to the manufacturer's instructions: Ratio <0.8: negative; Ratio ≥0.8 to <1.1: borderline; Ratio ≥1.1: positive. All samples with inconclusive results (i.e., borderline results with a ratio of ≥0.8 to <1.1) were excluded from the analysis. All testing of VZV IgG antibodies was conducted by a third party in a local private medical laboratory.

Data collection from the local private sector

We extracted VZV IgG antibody data at ProCare Medtech Laboratory – a local private medical laboratory in the Cheung Sha Wan area in Hong Kong. The medical laboratory is accredited by the International Organization for Standardization (ISO) 15189 quality management system under the Hong Kong Laboratory Accreditation Scheme (HOKLAS) provided by the Hong Kong Accreditation Service (HKAS). The laboratory mainly processes samples from medical diagnostic and health check-up centres that offer health check-ups and immigrant Visa medical examination services to the general public. We utilised the Laboratory Information System (LIS) installed in the laboratory's computers to generate PDF files for every tested year (2015, 2016, 2017, 2018, 2019, 2020). Each PDF file contains all the VZV IgG testing cases within the tested year, which includes the year of testing, sample identity (SID), laboratory identity (LID), patient's age, sex and results. All patient information was kept confidential, and the data extracted were anonymous to ensure confidentiality.

Data collection from the Centre for Health Protection (CHP)

Data on varicella notifications reported to the Department of Health from 1999 to 2020 was obtained by accessing the CHP website, which assessed the trends of varicella immunisation in the Hong Kong population. In addition, data on seroprevalence rates of VZV IgG antibodies listed on the CHP's website was used to evaluate the change in seroprevalence rates in the Hong Kong population after the universal varicella vaccination programme.

Statistical analysis

The testing data of VZV IgG antibodies obtained from the private medical laboratory were categorised at first into subgroups according to the patient's age, sex, year of sampling, and VZV IgG antibodies' testing results. The seroprevalence rates were calculated as a percentage (%) according to the following formula:

$$\frac{\text{Number of cases with positive VZV IgG result}}{\text{Number of cases measured}} \text{ X } 100 = \text{Seroprevalence rate (\%)}$$

With a 95% confidence interval for proportions, Pearson's Chi-square test was used for the univariate analysis and logistic model of the testing data. All data analysis was performed with Microsoft® Excel® (version 2107).

Results

Altogether, 3150 cases (689 males; 2461 females) were extracted from the LIS of the local private medical laboratory based on samples collected between 2015 and 2020 and analysed for the

presence of VZV IgG antibodies. The number of samples with positive VZV IgG antibodies was 2977; negative: 111; borderline: 62. These 62 samples with borderline VZV IgG antibodies were excluded from data analysis. The overall VZV seroprevalence in Hong Kong between 2015 – 2020 was 96.4% (95% CI: 95.8% – 97.1%).

VZV seroprevalence between 2015 and 2020

The seroprevalence rates of VZV IgG antibodies in the Hong Kong population between 2015 and 2020 are illustrated in Figure 1. There was virtually a decrease in the VZV seroprevalence rates over these years. With steeper VZV, IgG seroprevalence rates changed from 97.8% in 2015 to 96.7% in 2016, 96.9% in 2017, and 95.4% in 2018, respectively. The frequency of VZV IgG-positive samples decreased gradually from 95.4% in 2018 to 95.1% in 2020. However, evidence of overall statistical differences in the seroprevalence rates was not identified, X^2 (5, N = 3088) = 8.06, p > .05.

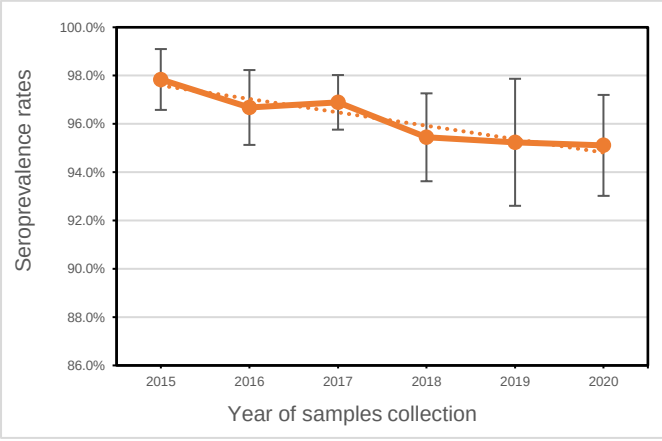

Figure 1. Seroprevalence rate of VZV IgG antibodies in the Hong Kong population based on samples collected between 2015 and 2020. Error bars represent 95% confidence intervals for proportions.

Gender-specific VZV seroprevalence

Figure 2 demonstrates that the seroprevalence rates of VZV IgG antibodies in the male population were generally lower than that of the female population in Hong Kong within the tested period between 2015 and 2020, except in 2018. After statistical calculation, there was a significant difference between the gender and the VZV seroprevalence rates within the tested period, X^2 (1, N = 3088) = 4.37, p = .037. Females were more likely than males to present immunity to VZV.

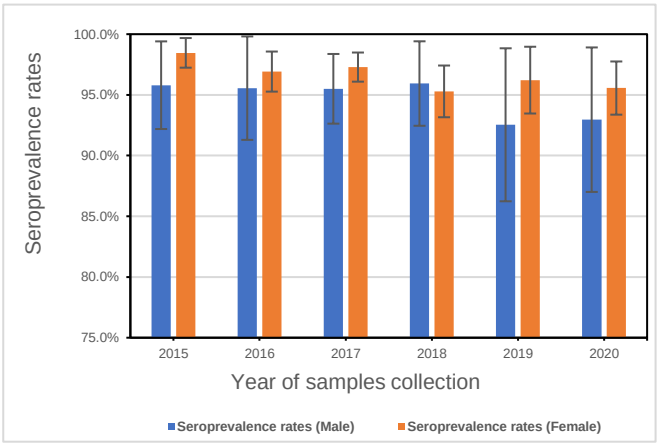

Figure 2. Gender-specific seroprevalence rates of VZV IgG antibodies in the Hong Kong population based on samples collected between 2015 and 2020. Error bars represent 95% confidence intervals for proportions.

Age-specific VZV seroprevalence

An age-specific seroprevalence rate of VZV IgG antibodies in the Hong Kong population according to five age groups (15 – 19, 20 – 24, 25 – 29, 30 – 34, 35 – 39) was illustrated in Figure 3a. The result indicates that the VZV IgG seroprevalence rates of the Hong Kong population increased with an increase in age group, with a steeper increase of VZV IgG seroprevalence rates from 73.2% in the age group of 15 – 19 to 91.3% in the 20 – 24 age group. Afterwards, the VZV IgG seroprevalence rates gradually increased from 94.8% in the 25-29 age

group to 96.3% in the 35-39 age group. Further statistical analysis discloses that there was a significant relationship between the age (15 – 39) and VZV IgG seroprevalence rates, X^2 (4, N = 1647) = 43.27, p < .001.

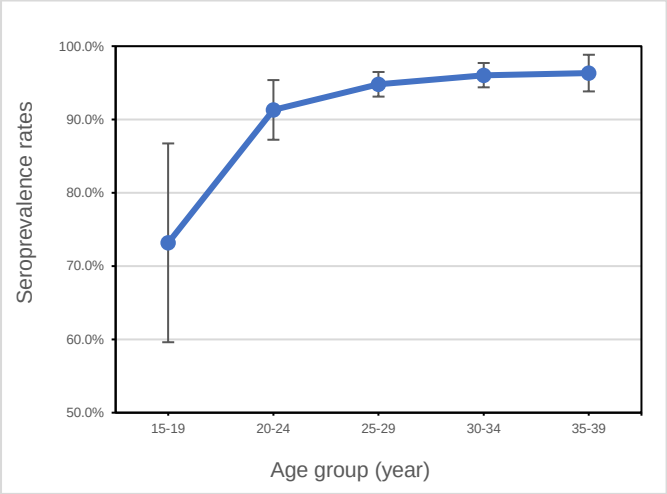

Figure 3a. Age-specific seroprevalence rates of VZV IgG antibodies in the Hong Kong population according to five age groups (15 – 19, 20 – 24, 25 – 29, 30 – 34, 35 – 39). Error bars represent 95% confidence intervals for proportions.

On the other hand, age-specific seroprevalence rates of VZV IgG antibodies in the Hong Kong population according to five age groups (40 – 44, 45 – 49, 50 – 54, 55 – 59, ≥60) indicates that the relationship between the age and VZV IgG seroprevalence rates was no longer significant among the older population equal to or greater than 40 years old (Figure 3b), X^2 (4, N = 1441) = 6.23, p = .183.

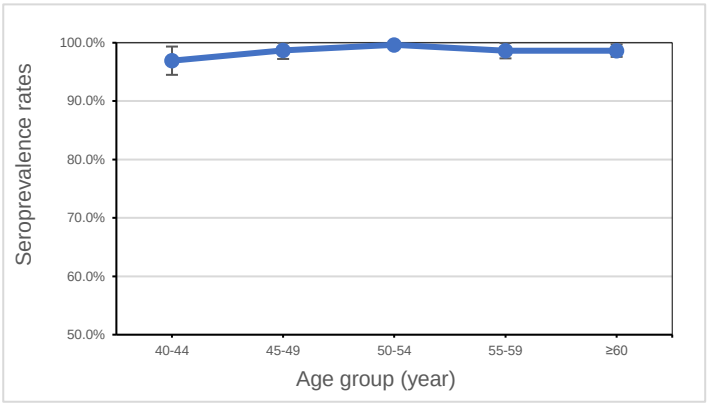

Figure 3b. Age-specific seroprevalence rates of VZV IgG antibodies in the Hong Kong population according to five age groups (40 – 44, 45 – 49, 50 – 54, 55 – 59, ≥60). Error bars represent 95% confidence intervals for proportions.

Discussions

In this study, the presence of VZV IgG antibodies in serum samples collected from the general population in Hong Kong between 2015 and 2020 was examined to assess the effect of implementing universal varicella vaccination in Hong Kong. Results indicate that 96.4% of tested individuals were VZV IgG seropositive. This rate is similar to the study by Fung et al. (2011) of VZV IgG seroprevalence among pregnant women in Hong Kong, with documented VZV IgG seroprevalence rates of 95.4%.[10] The high seroprevalence rates of VZV IgG in Hong Kong are probably associated with the high population density and close contact between kindergarten and school children promoting VZV transmission, resulting in more than 90% of the population varicella infections occurring by the age of 17 years.[11] Once infected with varicella, those individuals would obtain a long-lasting immunity to it, which may even last their lifetime. Therefore, there have been high seroprevalence rates of VZV IgG in Hong Kong's adult population. In addition, the study of Fung et al. (2011) has indicated that the higher the degree of urbanisation of a region, the

lower the mean age of varicella infection.[10] In Hong Kong, the mean age for varicella admission was 57.6 months, and the median age of varicella infection was six years[11,12], implying a remarkably high degree of urban development in Hong Kong.

VZV seroprevalence between 2015 and 2020

Between 2015 and 2020, there was no significant change in the VZV IgG seroprevalence rates in the Hong Kong population, and the results do not match the expectations proposed before the study. The primary reason for the unexpected results is probably the insufficient data in the younger group below the age of 15, so the immunity gained in children through the vaccination programme is not considered to influence the current study results. One possible reason for the insufficient data in the younger age group is that the testing data of VZV IgG antibodies generally came from the people who intended to identify the presence of immunity to VZV for obtaining Visa certification for immigration or international travel. Therefore, this study might not have data from the younger age group. This limitation may result in a biased reflection of the actual immunisation to VZV of the whole population, leading to the seemingly unchanged VZV IgG seroprevalence rates in the Hong Kong population during the tested period. Importantly, data from the younger age group is critical because they are most likely to be significantly affected by the universal varicella vaccination programme. Suppose the testing data of the younger population could be obtained. In that case, it might better illustrate the complete population immunisation to VZV, thereby allowing an unbiased prediction of the trend of varicella immunisation of the Hong Kong population after the universal varicella vaccination programme. In the current study, the size and distribution of data were unpredictable until it was extracted from the LIS of the local private medical laboratory, resulting in an unexpectedly low sample size from the younger population. To improve this, that is suggested to perform the VZV IgG seroprevalence study by collecting serum samples from a serum bank with a stratified sampling design (stratification by age), thereby allowing a sufficient sample size in the younger group aged 1 – 15 with a desirable sample number in each designated age groups. Despite this limitation, the results obtained from this study are still valid for assessing the trend of varicella immunisation and the change in seroprevalence rates of the adult population in Hong Kong. It is suggested that the VZV IgG seroprevalence rate in the adult population was not affected by the universal varicella vaccination programme as there was no significant alteration of the VZV IgG seroprevalences rates between 2015 and 2020 (Figure 1).

Although the data of VZV IgG seroprevalence in children aged 1 – 14 was excluded in this study, the data of varicella notifications from the CHP has disclosed that there has been a gradual decline of annual varicella notification rates, from 52.2% in 2015 to 45.3% in 2019[13], suggesting the benefits of the universal varicella vaccination in preventing varicella infections among children. In the United States, the numbers of infections, hospitalisations and deaths caused by varicella have dramatically declined after twenty years of implementing the varicella vaccination programme.[7] Over time, the universal varicella vaccination programme in Hong Kong is likely to achieve the expected benefits as in the United States.

Gender-specific VZV seroprevalence

This study showed relatively higher VZV IgG seroprevalence rates in the female population

compared with the male population (Figure 2). It seems the females in Hong Kong were more likely to present immunity to VZV, which does not match the expected results proposed before this study. One possible reason for this is that the varicella vaccination rates in the female population were higher than that of the male population in Hong Kong, implying the gender differences in attitudes and willingness towards vaccination. According to Akosionu *et al.* (2015) study on Asian Americans' attitudes towards HBV vaccination, females were four times more likely to get HBV vaccination than males, even though the male respondents were more likely to believe the effectiveness and benefits of HBV vaccination.[14] Similarly, females in Hong Kong are more likely to get varicella vaccination via the private market, resulting in relatively higher VZV IgG seroprevalence rates. Another possible reason would be sampling bias in the data collected, as the female samples account for 3/4 of the samples in this study. Ideally, the male-to-female (M: F) ratio of the samples collected should be close to the male/female distribution of the sampled population. In Hong Kong, the M: F ratio was approximately 1:1.19 in 2020[15], but the M: F ratio of the current study was 1:3.57, implicating the presence of oversampling in the female population and might result in a misrepresentation of the VZV IgG seroprevalence of the Hong Kong population. It is suggested that random samples from serum banks be drawn with a weighting adjustment by gender to match the Hong Kong population's M: F ratio. As a result, the results obtained could more accurately represent the tested population.

Age-specific VZV seroprevalence

The findings of age-specific VZV IgG seroprevalence rate from this study (Figure 3a, 3b) are consistent with age-specific VZV IgG seroprevalence data in 2015 listed on the CHP's website (VZV IgG seroprevalence rate at 15 – 19 years age group: 76%; 20 – 24: 92%; 25 – 29: 94%; 30 – 34: 100%; 35 – 39: 92%; >39: 98%)[16], suggesting that there was no significant difference in the VZV IgG seroprevalence study between public and private sectors. Both studies in VZV IgG seroprevalence indicate that in Hong Kong, more than 70% of individuals acquire immunity to VZV before reaching adulthood. However, this rate was much lower than those of Western countries with the implementation of universal varicella vaccination, like Italy (>89%) and the United States (>90%).[17,18] The reasons for the lower rate in Hong Kong are unclear, but this may be associated with differences in the pattern of child care or the shorter period of implementing the universal varicella vaccination programme. In addition, this study shows a noticeable relationship between age and VZV IgG seroprevalence rates in the younger age group, especially in the group below 25 years of age (Figure 3a). As for the age-specific VZV IgG seroprevalence data from CHP, increasing the age to acquire VZV IgG antibodies in people 1 – 24 years of age suggests the primary force of VZV transmission occurred in the younger population in Hong Kong.

Apart from this, the data of age-stratified seroprevalence rates of VZV antibodies from the CHP has disclosed that the VZV IgG seroprevalence at 1 – 4 years of age increased dramatically from 26% in 2010 to 36% in 2015 after a year of implementing the universal varicella vaccination programme[16], suggesting the importance of varicella vaccination in providing VZV immunity to children. Although the data of VZV IgG seroprevalence at 1 – 4 years of age between 2016 and 2020 was absent in this study or the public sector, we would expect that the VZV IgG seroprevalence rates at 1 – 4 years of

age would be increased year by year until reaching the highest possible VZV IgG seroprevalence rates.

In conclusion, this study confirms a high VZV IgG seroprevalence rate among the Hong Kong population, with 96.4% overall VZV IgG seroprevalence and more than 70% of individuals living in Hong Kong acquire VZV immunity before reaching adulthood, probably resulted from the high degree of urban development and population density in Hong Kong. There was no significant change in the VZV IgG seroprevalence rate among the adult population in Hong Kong. Gender differences in VZV IgG seroprevalence revealed a relatively higher VZV IgG seroprevalence in the female population than that of the male population, probably due to the greater willingness towards varicella vaccination in the female population or the presence of oversampling in the female population. This study's data on age-specific VZV seroprevalence rates are consistent with the age-specific VZV IgG seroprevalence rates from the public sector in Hong Kong. A significant relationship is identified between age and VZV IgG seroprevalence in the population below 25, indicating the higher prevalence of varicella among younger people in Hong Kong. The data of VZV seroprevalence from the CHP showed a dramatical increase of VZV IgG seroprevalence rates in the 1 – 4 age group and a gradual decline of annual varicella notification rates after implementation of the universal varicella vaccination programme, suggesting the importance and benefits of the vaccination programme in providing VZV immunity to and preventing varicella infections among children in Hong Kong.

References

1. Papaloukas O, Giannouli G and Papaevangelou V. Successes and challenges in varicella vaccine. Therapeutic Advances in Vaccines. 2014; 2(2):39-55.
2. Arvin AM, Moffat JF, Sommer M, et al. Varicella-zoster virus T cell tropism and the pathogenesis of skin infection. Current Topics in Microbiology and Immunology. 2010; 342:189-209.
3. Huch JH, Cunningham AL, Arvin AM, et al. Impact of varicella-zoster virus on dendritic cell subsets in human skin during natural infection. Journal of Virology. 2010; 84(8):4060-4072.
4. Ku CC, Zerboni L, Ito H, et al. Varicella-zoster virus transfer to the skin by T Cells and modulation of viral replication by epidermal cell interferon-alpha. The Journal of Experimental Medicine. 2004; 200(7):917-925.
5. Lin W, Chang C, Ko M and Lin, S. A fatal case of severe systemic varicella zoster infection in a patient with chronic use of immunosuppressive agents for cutaneous vasculitis. IDCases. 2020; 19:e00667.
6. World Health Organization. Varicella and herpes zoster vaccines: WHO position paper, 2014. Available: https://www.who.int/wer/2014/wer8925.pdf [Accessed 28 June 2021].
7. Hamborsky J, Kroger A and Wolfe C. Epidemiology and prevention of vaccine-preventable diseases, 13th Edition 2015. Washington, D.C.: U.S. Dept. of Health & Human Services, Centers for Disease Control and Prevention.

8. Marin M, Watson, TL, Chaves SS, et al. Varicella among adults: data from an active surveillance project, 1995-2005. The Journal of Infectious Diseases. 2008; 197(s2):S94-S100.

9. Centre for Health Protection, Department of Health, The Government of the Hong Kong Administrative Region. Scientific committee on vaccine-preventable diseases recommendations on the use of varicella (Chickenpox) vaccine in Childhood Immunisation Programme, 2013. Available: http://www.chp.gov.hk/files/pdf/recommendations_on_the_use_of_varicella_vaccine_in_cip_r.pdf [Accessed 29 May 2021].

10. Fung L, Lao T, Suen S, et al. Seroprevalence of varicella-zoster virus among pregnant women in Hong Kong: comparison with self-reported history. Vaccine. 2011; 29(46):8186-8188.

11. Chan J, Tian L, Kwan Y, et al. Hospitalisations for varicella in children and adolescents in a referral hospital in Hong Kong, 2004 to 2008: a time-series study. BMC Public Health. 2011; 11(1).

12. Chan DYW, Edmunds WJ, Chan HL, et al. The changing epidemiology of varicella and herpes zoster in Hong Kong before universal varicella vaccination in 2014. Epidemiology and Infection. 2018; 146(6):723-734.

13. Centre for Health Protection, Department of Health, The Government of the Hong Kong Administrative Region. Statistics on communicable diseases - number of notifiable infectious diseases by month, 2020. Available: https://www.chp.gov.hk/en/static/24012.html [Accessed 8 July 2021].

14. Akosionu O, Virnig B, Call K, et al. The impact of gender differences in attitudes and beliefs concerning HBV vaccination and screening in the Lao community. Journal of Immigrant and Minority Health. 2015; 18(1):277-281.

15. Census and Statistics Department, The Government of the Hong Kong Administrative Region. Women and men in Hong Kong – key statistics, 2021. Available: https://www.censtatd.gov.hk/en/data/stat_report/product/B1130303/att/B11303032021AN21B0100.pdf [Accessed 20 August 2021].

16. Centre for Health Protection, Department of Health, The Government of the Hong Kong Administrative Region. Statistics on laboratory surveillance – serological surveillance: seroprevalence rates of varicella-zoster virus antibodies,2015. Available: https://www.chp.gov.hk/en/statistics/data/10/641/701/3691.html [Accessed 8 July 2021].

17. De Donno A, Kuhdari P, Guido M, et al. Has VZV epidemiology changed in Italy? Results of a seroprevalence study. Human Vaccines & Immunotherapeutics. 2016; 13(2):385-390.

18. Reynolds M, Kruszon-Moran D, Jumaan A, et al. Varicella seroprevalence in the U.S.: data from the national health and nutrition examination survey, 1999–2004. Public Health Reports. 2010; 125(6):860-869.

Association of Thyroid hormones and fasting glucose and HbA1c: A retrospective study of Hong Kong's general population from a single private centre for five years.

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Abstract

Thyroid hormone is essential in regulating metabolism, e.g. glucose and lipid metabolism. T3 and T4 are primary hormones secreted by thyroid glands, and the pituitary gland's response to the plasma level of thyroid hormone secretes TSH. Fasting glucose and haemoglobin A1c are generally correlated and affected by different diseases. As thyroid hormone regulates metabolism, this study assesses any correlation between thyroid hormone and fasting glucose and HbA1c. Besides assessing the general correlation, correlation is also assessed after adjustment of gender and age group.

A retrospective study was conducted. Twenty-four thousand five hundred seventy-eight data sets from 2013 to 2020 were used for analysis, including 14157 females (58%) and 10421 males (42%). The age range is from 1 year old to 99 years old. Correlation between thyroid hormone and fasting glucose and HbA1c was analyzed using Pearson correlation according to the overall picture of all selected data, gender and age groups. Differences between age and gender were analyzed using one-way ANOVA and independent t-test, respectively.

Strong correlations and weak correlations are found in this study. A strong correlation is found between free T4 and fasting glucose in young subjects. Meanwhile, a general positive correlation between TSH and HbA1c was found for weak correlations. A negative correlation between free T4 and fasting glucose is observed in male subjects. A positive correlation between TSH and HbA1c and a negative correlation between free T4 and HbA1c were observed in the age group 18 to 30 years old. Significant differences are found within all analytes of different age groups and in free T4, TSH, and fasting glucose between genders.

The correlation between thyroid hormone fasting glucose and HbA1c could differ in genders and ages. Since a significant correlation is found in young subjects, it is suggested that they have closer monitoring of thyroid hormone, glucose, and HbA1c levels to have an early diagnosis of diabetes and thyroid dysfunction. Gender and age differences, lifestyle and underlying diseases were believed to contribute to the different correlations observed. Limitations in data selection contribute to inconsistency of findings; it will be better to expand the selection criteria to identify other factors, e.g. smoking, pregnancy, medication, etc., that affect thyroid hormone and fasting glucose and HbA1c.

Keywords: *thyroid hormone, free T4, TSH, haemoglobin A1c, fasting glucose*

Introduction

The thyroid hormone plays a vital role in maintaining metabolism. It regulates metabolism control, body temperature and body weight. Thyroxine (T4) and triiodothyronine (T3) are the two main thyroid hormones secreted by the thyroid gland [1]. The hypothalamus secretes a thyrotropin-releasing hormone (TRH) and stimulates the pituitary gland to secrete thyroid-stimulating hormone (TSH). TSH act on the thyroid gland to stimulate secretion of T3 and T4 as required. Increased thyroid hormone levels inhibit TRH and TSH production, forming a negative feedback loop [2].

Thyroid function tests measure TSH, T3 and T4 levels to assess thyroid dysfunction. Most clinicians rely on TSH and free T4 (FT4) to assess thyroid function. FT4 is used instead of total T4 because bound T4 included in total T4 cannot be used by tissue cells since TSH stimulates the thyroid to produce more T4 and T3. The levels of T4 and T3 are then inversely related to TSH levels. FT4 level can be used as a proxy of T3 level in serum [3]. Therefore, TSH and FT4 are selected as target analytes in this study.

Patients with impaired thyroid function have a higher risk of developing obesity or other metabolic syndromes [4]. TSH and free T3 serum levels were positively related to metabolic syndrome [5]. Studies suggest that TSH level is positively related to type 2 diabetes. Moreover, patients affected by prediabetes have a higher risk of nephropathy and cardiovascular disease, and impaired glucose may bring pathogenic effects on prediabetes patients. Overt thyroid dysfunction has an alteration in glucose levels. However, the effect of glucose homeostasis, the balance of insulin and glucagon to maintain blood glucose, by thyroid hormone within the reference range is unknown [6]. This study focuses on the effect of thyroid hormone within the normal range influencing blood glucose.

Glucose enters circulation mainly through daily diet absorption, glycogenolysis and gluconeogenesis by the liver. Globally, neuropeptide regulation achieves glucose homeostasis and supports life [7]. Fasting glucose and haemoglobin A1c (HbA1c) are generally considered glucose homeostasis parameters.

HbA1c is a marker of long-term glycaemic control and is commonly used as a diagnostic indicator of prediabetes and diabetes—higher ambient glucose results in a higher HbA1c level. Patients with insulin resistance or diabetes tend to have a higher HbA1c level. However, HbA1c level is affected according to red blood cell life; hyperthyroidism shortens red blood cell life, while hypothyroidism is the opposite [6,8].

The thyroid hormone is closely related to glucose homeostasis; however, research on how glucose homeostasis changes in response to thyroid hormone levels needs consistency [6]. Thyroid hormone is related to pancreatic β-cell development and affects glucose metabolism by influencing different organs, e.g. liver, skeletal muscle and central nervous system [7]. Thyroid hormones increase gastrointestinal motility to promote glucose absorption. It increases glucose levels by increasing gluconeogenesis and glycogenolysis in the liver. Hyperthyroidism increases glucose consumption in skeletal muscles [7]. As there are different mechanisms for thyroid hormones to be involved in glucose metabolism and production, it is therefore important to study the effect of thyroid hormones on glucose homeostasis index, e.g., fasting glucose and HbA1c.

Hypothyroidism

Patients with hypothyroidism have a reduction of gluconeogenesis in skeletal muscle and adipose tissue, with impaired glycogenolysis. Thyroid hormone and insulin are interrelated with the production of stress hormone cortisol. Reduced cortisol levels result in hypoglycaemia [7].

Hyperthyroidism

Hyperthyroidism increases metabolic rate, thus increasing the elimination rate of insulin from circulation, resulting in increased blood glucose and vice versa. However, increased thyroid hormone also stimulates insulin secretion. Excess thyroid hormone increases the glucose level in peripheral blood through different mechanisms [7]. Long-term hyperglycemia impairs the peripheral deiodination of T4 to T3, causing thyroid dysfunction. Patients with diabetes and hyperglycaemia are thus closely related to an increased risk of thyroid dysfunction [9].

Insulin resistance and dysglycaemia persist in both hypothyroidism and hyperthyroidism. Hyperthyroidism increases the recruitment of glucose transporters and increases glucose disposal in peripheral tissue; however, when there is an increase in glucose output, glucose absorption, and reduced muscle glycogen storage, the above regulation may be overruled [10].

Gender differences

Females have a significantly higher risk of thyroid dysfunction [5]. Men are more prone to impaired fasting glucose, while males and females have similar HbA1c levels; this suggests that glucose metabolism is similar in different genders [11]. Sex hormones may contribute to the difference in glucose homeostasis.

Age and thyroid hormone

Hypothyroidism incidences increase with age and increase in females [7]. Fasting glucose increases with age [12]. An increase in plasma glucose levels may be due to the resistance to insulin or impaired secretion of insulin. Ageing is said to be one of the reasons causing such impairment. HbA1c also increases according to age. Serum TSH levels decrease with age, while free T4 levels remain unchanged [13].
Since thyroid hormones are closely related to the metabolism and production of glucose, this study aims to study and identify the association between thyroid hormones, fasting glucose, and HbA1c. If thyroid hormone is confirmed to influence fasting glucose and HbA1c significantly, people should focus more on thyroid function when monitoring blood glucose, especially for diabetes patients.

Methodology

Data were collected from the health check group of a private HOKLAS-accredited laboratory to evaluate the relationship between thyroid hormone, fasting glucose, and HbA1c. This study selected target FT4, TSH, fasting glucose, and HbA1c analytes.
A dry lab was applied in this retrospective study with data from 2013 to 2020. No follow-up of subjects and no extra patient control are required in a retrospective study. Since the data collected are not designed for the study, there may be limitations on interpretation and data selection. Since this is a retrospective study, there are no extra steps for patient preparation, and patient health history will not be applicable.

Statistical software SPSS and Microsoft Excel were used for data processing, including sorting data and analyzing using statistical methods to study any association among different aspects.
Pearson correlation was used to analyze the correlation between thyroid hormone fasting glucose and HbA1c. Pearson correlation coefficient (r) was used to indicate the strength and direction of a linear relationship. One-way ANOVA analysis was used to analyze differences between different age groups in each analyte. An independent t-test was used to investigate differences between genders.
TSH and FT4 are the target hormones in this study against fasting glucose and HbA1c. Different genders and ages are found to increase the risk of thyroid dysfunction, and gender and age differences are found in target analytes; this study will also investigate any influence of thyroid hormone on fasting glucose and HbA1c regarding gender and age. Data were sorted according to age and gender

to study whether there is a relationship between gender or age group change with thyroid hormone levels towards fasting glucose and/or HbA1c. Age range cover and the number of data sets available depending on the patients undergoing laboratory testing of target analytes in the past few years. The age was generally divided into decades, except for the first two groups, where the first group account for all subjects below 18 years old; other age groups are 18 to 30 years old, 31 to 40 years old, 41 to 50 years old, 51 to 60 years old, 61 to 70 years old and 71 years old or above.

This study mainly focuses on data sets with normal thyroid hormones to understand whether the increase in thyroid hormone affects fasting glucose and HbA1c levels. People with high or low thyroid hormone may be under medication, affecting the interpretation of results. Since no medication history was available in this study, excluding data outside the reference range can limit the influence.

In the data selection steps, data sets are also analyzed for distribution. To obtain a reliable data set, outliers of different analytes are excluded to provide a generally normal distributed data set. This data set are said to be with limited bias increasing validity of the study.

Analyzer and reference range used in the laboratory:

	TSH	Free T4	Glucose, Fasting	HbA1c
Analyzing instrument	CMIA by Abbott Alinity i system		HPLC by Sysmex Tosoh Automated Glycohemoglobin Analyser HLC-723 G11	CMIA by Abbott Alinity c system
Reference range	Euthyroid, non-pregnant 0.35 - 2.82 mIU/L	● Euthyroid, non-pregnant: 9.01-19.05 pmol/L ● 0-<1Y: 10.90-23.6 pmol/L ● 1-5Y: 11.07-20.85 pmol/L ● 6-10Y: 10.81-18.92 pmol/L	Normal: 3.9 - 5.5 mmol/L Impaired: 5.6 - 6.9 mmol/L Diabetes mellitus (DM): >/= 7.0 mmol/L	Non-diabetic range (>18 years): 4.0 - 6.0% Increased risk: 5.7 - 6.4% Diabetes: >or= 6.5 %

Results

Data from 2013 to 2020 were collected, including 51912 females (42%) and 70760 males (58%), ranging from 1 year old to 101 years old. Since different patients have different panels of health checks, the available data may differ in different subjects.

Subjects with thyroid dysfunction could lead to pathological alteration in metabolism, and thus, to provide a more representative figure, average free T4 and TSH results are selected as inclusion criteria, while those outside the reference range are excluded. Since diabetes mellitus may have different extent of influence on target analytes, subjects with results classified as diabetes mellitus are excluded, i.e. fasting glucose levels ≥ 7 mmol/L or HbA1c ≥ 6.5%. Although there are particular reference ranges in FT4 for patients below ten years old, all those results are within both reference ranges for below ten years old and above ten years old, and no specific exclusion steps were performed for results from subjects below ten years old.

After exclusion, 24578 data sets were used for analysis, including 14157 females (58%) and 10421 males (42%). The age range is from 1 year old to 99 years old. Seven thousand seven hundred fifty-three free T4 results, 22620 TSH results, 16694 fasting glucose, and 2587 HbA1c results are available.

Data are analyzed using Pearson correlation of SPSS; that correlation significant in 0.05 confidence interval are labelled with *, while those significant in 0.01 level are labelled with ** in the following tables and highlighted to facilitate reading. Significant levels and

sample sizes are included in the following tables.

Table 1 shows a negative correlation (r = 0.046) between TSH and HbA1c, which is statistically significant (p = 0.032) between analytes, without adjustment for age and gender. After gender adjustment, there is no significant finding between analytes in female subjects. A negative correlation is observed between FT4 and fasting glucose in male subjects, giving a r=0.059 and p-value 0.048, which is statistically significant. In the age group below 18, a high positive correlation between FT4 and fasting glucose is observed (r = 0.682, p < 0.01). The correlation is highly significant with sample size 31. The higher the FT4 level, the higher the fasting glucose level in these young subjects. In the age group 18 to 30, a negative relationship (r=-0.333) is observed between FT4 and HbA1c, with a high significance of p-value <0.01. A positive relationship (r=0.186) is observed between TSH and HbA1c with a significance of p-value <0.05. The sample size for these two relationships is 67 and 121, respectively. There is no significant correlation between thyroid hormone and fasting glucose and HbA1c in other age groups.

ANOVA is used to analyze differences in each analyte between different age groups. Table 2 shows significant differences (p-value < 0.01) in different age groups in all four target analytes. Line graphs demonstrate the relationship between thyroid hormone and fasting glucose or HbA1c. Fasting glucose and HbA1c increase with age (Figure 1 to 4). Thyroid hormone effect on fasting glucose and HbA1c are not found except in the age group below 18, in which an increase in FT4 level increase fasting glucose levels (Figure 1) is already observed in Pearson correlation analysis.

Independent t-test is used to analyze any differences in each analyte between genders. Table 3 shows that the p-value of Levene's test is >0.05 in FT4, equal variances for FT4 are assumed, the p-value of Levene's test in TSH, fasting glucose, and HbA1c is <0.05, equal variances are not assumed for the three analytes. By referring to the corresponding p-value in the t-test, there was no significant difference in HbA1c between the two genders, with p-value = 0.408, >0.05. At the same time, a significant difference was found between FT4, TSH and fasting glucose, with p-value < 0.01.

Line graphs are used to demonstrate the correlation and differences of thyroid hormone and fasting glucose or HbA1c between genders. The mean fasting glucose levels of males are higher than those of females in all ranges of FT4 and TSH (Figure 5, 6). The mean HbA1c levels show no apparent difference between males and females in TSH levels below or equal to 2.35mIU/L (Figure 7, 8). In comparison, HbA1c levels increased sharply in males in the TSH range above 2.35mIU/L and significantly higher than in females. The mean HbA1c levels show no apparent difference between males and females in FT4 levels ≤17 pmol/L, but the HbA1c level increased sharply in the males in the FT4 range >17 pmol/L and was higher than in females.

Correlations		overall correlation		Female		Male		< 18 years old		18 - 30 years old	
		FT4	TSH	FT4	TSH	FT4	TSH	FT4	TSH	FT4	TSH
FGLU	Pearson Correlation	-0.009	-0.007	0.014	0.014	-.059*	-0.011	.682**	0.013	-0.014	0.051
	Sig. (2-tailed)	0.643	0.359	0.548	0.212	0.048	0.33	0	0.906	0.845	0.053
	N	2913	15916	1784	7806	1129	8110	31	86	195	1443
HbA1c	Pearson Correlation	-0.021	.046*	0.023	0.037	-0.074	0.058	-0.178	0.231	-.333**	.186*
	Sig. (2-tailed)	0.47	0.032	0.543	0.236	0.104	0.056	0.579	0.427	0.006	0.041
	N	1158	2126	680	1026	478	1100	12	14	67	121

		31 - 40 years old		41 - 50 years old		51 - 60 years old		61 - 70 years old		> 70 years old	
		FT4	TSH	FT4	TSH	FT4	TSH	FT4	TSH	FT4	TSH
FGLU	Pearson Correlation	0.04	-0.007	-0.026	-0.017	0.03	-0.021	-0.07	-0.035	0.088	-0.032
	Sig. (2-tailed)	0.418	0.653	0.475	0.234	0.389	0.19	0.139	0.212	0.16	0.475
	N	415	3637	728	5138	826	3747	452	1311	259	489
HbA1c	Pearson Correlation	-0.032	0.005	0.026	-0.016	-0.049	0.024	-0.003	0.067	0.174	0.039
	Sig. (2-tailed)	0.71	0.924	0.678	0.717	0.365	0.572	0.962	0.24	0.055	0.613
	N	138	371	261	523	348	568	209	313	122	167

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 1. Correlation FT4 and TSH against fasting glucose and haemoglobin A1c concerning gender and age groups using Pearson correlation analysis.

➢ ANOVA analysis according to age groups

		Sum of Squares	df	Mean Square	F	Sig.
FT4	Between Groups	154.000	6	25.667	9.401	0.000
	Within Groups	21108.044	7731	2.730		
	Total	21262.043	7737			
TSH	Between Groups	7.977	6	1.330	4.748	0.000
	Within Groups	6309.824	22535	0.280		
	Total	6317.801	22541			
FGLU	Between Groups	541.574	6	90.262	383.966	0.000
	Within Groups	3907.250	16621	0.235		
	Total	4448.824	16627			
HbA1c	Between Groups	64.255	6	10.709	76.346	0.000
	Within Groups	355.029	2531	0.140		
	Total	419.284	2537			

Table 2. One way ANOVA shows any significant differences between age groups is

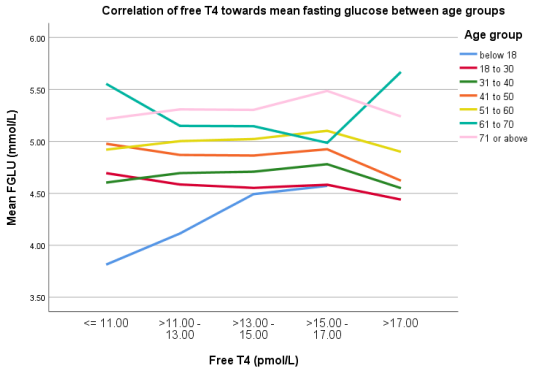

Figure 1. Correlation of free T4 levels towards mean fasting glucose levels between age groups.

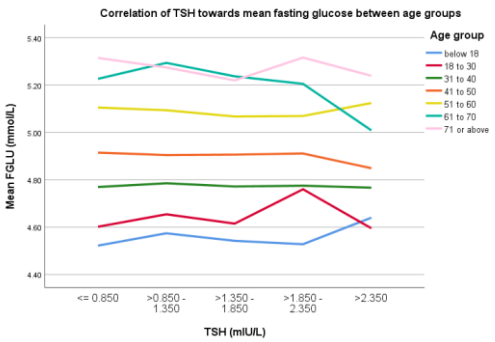

Figure 2. Correlation of TSH levels towards mean fasting glucose levels between age groups.

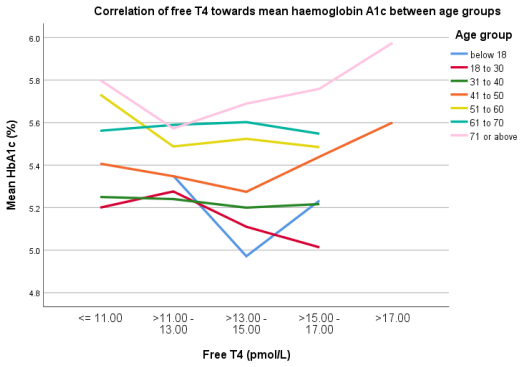

Figure 3. Correlation of free T4 levels towards mean haemoglobin A1c levels between age groups

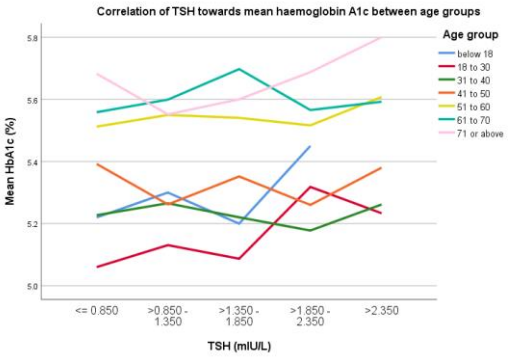

Figure 4. Correlation of TSH levels towards mean haemoglobin A1c levels between age groups.

➢ Independent t-test analysis according to gender

Group Statistics

	Gender	N	Mean	Std. Deviation	Std. Error Mean
FT4	F	5561	13.3024	1.66795	.02237
	M	2192	13.3974	1.64006	.03503
TSH	F	12786	1.18097	.551146	.004874
	M	9834	1.15476	.499673	.005039
FGLU	F	8266	4.8303	.49045	.00539
	M	8428	5.0387	.52243	.00569
HbA1c	F	1303	5.425	.3787	.0105
	M	1284	5.438	.4316	.0120

Table 3. Independent T-test compares the mean difference between genders.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
Independent Samples Test		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
FT4	Equal variances assumed	.419	.517	-2.267	7751	.023	-.09491	.04187	-.17698	-.01284
	Equal variances are not assumed.			-2.284	4074.814	.022	-.09491	.04156	-.17639	-.01343
TSH	Equal variances assumed	131.708	.000	3.692	22618	.000	.026215	.007100	.012298	.040133
	Equal variances are not assumed.			3.739	22017.676	.000	.026215	.007010	.012474	.039956
FGLU	Equal variances assumed	26.413	.000	-26.558	16692	.000	-.20837	.00785	-.22375	-.19300
	Equal variances are not assumed.			-26.574	16660.194	.000	-.20837	.00784	-.22374	-.19301
HbA1c	Equal variances assumed	12.937	.000	-.828	2585	.408	-.0132	.0160	-.0445	.0181
	Equal variances are not assumed.			-.827	2531.918	.408	-.0132	.0160	-.0445	.0181

Table 4. Independent T-test compares the mean difference between genders.

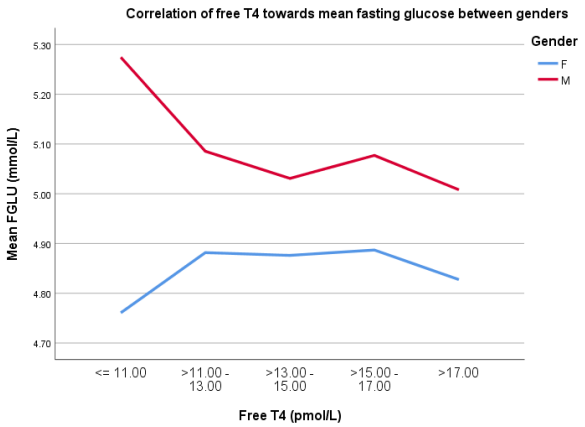

Figure 5. Correlation of free T4 level towards mean fasting glucose levels between genders.

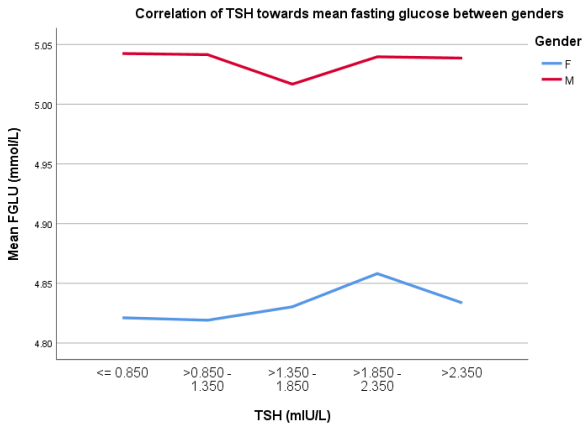

Figure 6. Correlation of TSH levels towards mean fasting glucose levels between genders.

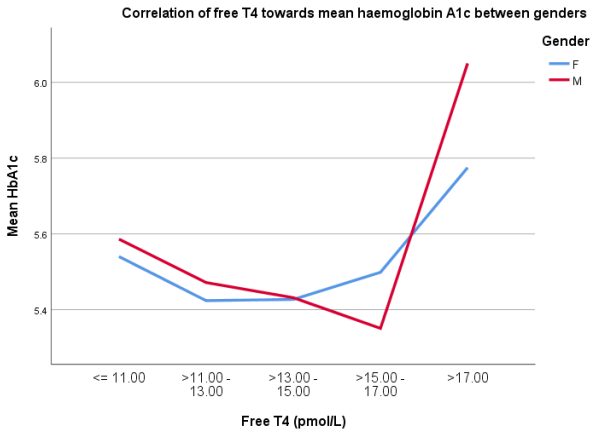

Figure 7. Correlation of free T4 levels towards mean haemoglobin A1c levels between genders.

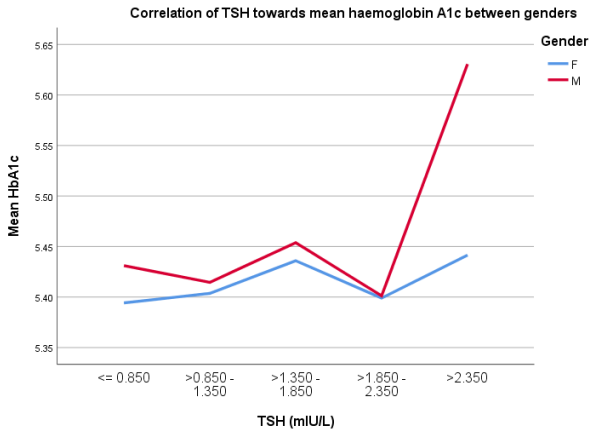

Figure 8. Correlation of TSH levels towards mean haemoglobin A1c levels between genders.

Discussions

Thyroid hormones stimulate insulin secretion, glucagon release, and glucose absorption. Controlling thyroid hormone levels can influence glucose absorption and plasma glucose to a certain extent. In this study, we hypothesize that thyroid hormone will positively correlate with fasting glucose and HbA1c.

Besides studying the general correlation between thyroid hormone and fasting glucose and HbA1c, this study also focused on whether thyroid hormone influences fasting glucose and HbA1c in the same way within females, males, and different age groups. One strong correlation and several weak correlations were found.

For the strong correlation, increased FT4 levels increase fasting glucose levels in people under 18. People under 18 years old with hyperthyroidism may pose a higher risk of developing high blood glucose. As this correlation is not demonstrated in other age groups, glucose levels are more likely to be affected by thyroid hormone in young subjects. Young people with hyperthyroidism are suggested to have a closer monitoring of blood glucose. On the other hand, those who have high blood glucose should also check for

thyroid function to have an early diagnosis of thyroid dysfunction. Although a strong correlation was found, the sample size is small; recruiting more subjects under 18 years old is recommended to perform a focusing study and provide a more accurate figure.

Some statistically significant but weak correlations were also demonstrated. A positive relationship is observed between TSH and HbA1c, showing that an increase in TSH level may decrease HbA1c. Regarding gender, a negative relationship between FT4 and fasting glucose was observed only in males but not in females. This finding contradicts Kim et al. [14], where a positive relationship was found in female and male subjects instead. This contradiction may be because adjustment with age was not made in this study when analyzing the correlation concerning gender. Last, although there is a statistically significant correlation between HbA1c and FT4 or TSH, the correlation is weak, and the r-value is close to zero; we believe that the correlation observed is due to other factors instead of the effect of thyroid hormone. Different factors are believed to contribute to limitations in this study. Since there is no unique selection of patients, the results may be affected by those risk factors.

Smoking

Smoking may exert different effects on thyroid glands, affecting thyroid hormone levels. The TSH levels may be decreased, and T3 and T4 levels may increase [15]. Former smokers are found to have similar TSH levels after a period of smoking cessation compared to non-smokers. This shows that the effect of smoking on TSH and thyroid hormone is reversible. Wang et al. [16] stated that a significant increase in fasting glucose is found in heavy smokers. For moderate and above smokers, there is an increase in HbA1c and a decrease in plasma insulin.

Pregnancy

Pregnant women produce more thyroid hormones during early pregnancy to supply suitable thyroid hormones to themselves and the fetus. This increases serum FT4 levels and decreases the TSH level in early pregnancy [17].

Medical consideration

Some of the medicines may affect glucose levels. Diabetes patients under treatment may not have been excluded from the study. The fasting glucose and HbA1c levels changes may be due to drug effects instead of thyroid hormone.

HbA1c may change under conditions that shorten the red blood cells' life span, e.g. haemolytic anaemia, splenomegaly, drug effects, etc. [18]. Patients may have a low HbA1c level even if there is a high fasting glucose level under drug-induced haemolysis.

Liver disease

Chronic liver disease is accompanied by insulin resistance and beta cell dysfunction, thus affecting glucose levels. Patients with chronic liver disease have complications of anaemia, which in turn have shortened red blood cell life span and thus affect HbA1c level [19].

Medical history and lifestyle are not collected in this study; we cannot identify any influence of factors other than age and gender on target analytes level. The above provides a possible explanation of this study's weak correlation and contradiction.

We also investigate whether there is a difference between gender and age groups to see if extra factors affect the levels of each analyte. Concerning gender, there is a significant difference in FT4, TSH and fasting glucose. Sexual hormones affect thyroid hormone levels to a certain extent. In estrogen dominance, the liver increases the production of thyroid-binding globulin, which releases

and binds to free T3 and FT4 in circulation, affecting thyroid hormone function. Progesterone significantly increased FT4 levels [20]. This suggests that sex hormones contribute to differences between genders. Mauvais-Jarvis [11] suggested that the HbA1c level is identical between males and females; this study agrees with this finding. No difference is found with HbA1c between genders, suggesting the postprandial glucose excursion is similar among genders [11]. The difference in glucose levels between genders should be due to differences in height and muscle mass and associated glucose consumption.

Concerning age groups, differences are found in all analytes. Fasting glucose and HbA1c levels increase with age, while no correlation between fasting glucose and HbA1c is observed in subjects >30 years old. Increasing fasting glucose and HbA1c levels in older subjects should be due to factors other than thyroid hormone. An increase in plasma glucose level results from production and metabolic disorder, possibly due to insulin resistance or impaired insulin secretion. Increasing age is often accompanied by increased adiposity, medication, and decreased exercise, and therefore, is a risk factor for insulin resistance and reduces the efficacy of insulin production. People should focus more on glucose homeostasis with increasing age to reduce the risk of developing diabetes mellitus. Different factors, such as lifestyle, are associated with thyroid hormones. It is better to develop a healthy lifestyle to limit the effect of alteration of thyroid hormone levels. Limiting thyroid hormone level alteration can reduce the effect of thyroid hormone on plasma glucose levels, reducing the risk of developing diabetes mellitus.

Fasting glucose and HbA1c levels are generally agreed to be correlated; it is hypothesized that thyroid hormone will affect fasting glucose and HbA1c similarly; however, in this study, thyroid hormone does not influence fasting glucose and HbA1c to the same extent. It is suggested to be due to limitations on data collection. As this is a retrospective study, most of the data sets collected do not contain all four target analytes in this study. Correlation between thyroid hormone and fasting glucose or HbA1c may not be generated from the same subject; subject variation contributes to variation in the test result, and thus, the finding of thyroid hormone against fasting glucose and haemoglobin A1c may not be comparable.

Due to the inconsistency among the studies, we believed there are factors other than thyroid hormone affecting fasting glucose level and HbA1c level. Although some of the findings agree with other studies, a more comprehensive study is required to confirm whether the correlation is due to thyroid hormone or other factors since different factors affect our target analytes to a certain extent.

Conclusion

Although a positive correlation is not observed as assumed, a strong positive correlation in subjects below 18 years old agrees with the hypothesis. This study suggests that patients with thyroid dysfunction should also monitor for metabolic disorders since thyroid hormones are found to have correlation with glucose and HbA1c. On the other hand, patient with diabetes or hypoglycaemia can also check for thyroid function since thyroid hormone take an important role in metabolism. To obtain a more comprehensive and representative database, future studies can expend the selection criteria in subject selection to provide the opportunity to include more diverse groups of subjects to identify factors affecting correlation between thyroid hormone and fasting glucose and HbA1c. It can focus more on statistically significant findings obtained in this study with a larger sample size.

References

1. Shahid MA, Ashraf MA, Sharma S. Physiology, thyroid hormone. StatPearls; 2022.
2. Gauthier BR, et al. Thyroid hormones in diabetes, cancer, and aging. Aging Cell. 2020;19: e13260. Available from: https://doi.org/10.1111/acel.13260
3. Armstrong M, Asuka E, Fingeret A. Physiology, thyroid function. StatPearls; 2023.
4. Pacifico L, Anania C, Ferraro F, Andreoli GM, Chiesa C. Thyroid function in childhood obesity and metabolic comorbidity. Clin Chim Acta. 2012;413:396-405. Available from: https://doi.org/10.1016/j.cca.2011.11.013
5. Meng Z, et al. Gender and age impacts on the association between thyroid function and metabolic syndrome in Chinese. Medicine (United States). 2015;94.
6. Ha J, et al. Association of serum free thyroxine and glucose homeostasis: Korea National Health and Nutrition Examination Survey. Korean J Intern Med. 2021;36(Suppl):S170.
7. Eom YS, Wilson JR, Bernet VJ. Links between thyroid disorders and glucose homeostasis. Diabetes Metab J. 2022;46:239.
8. Bhattacharjee R, et al. Effects of thyroid status on glycated hemoglobin. Indian J Endocrinol Metab. 2017;21:26.
9. Kapadia KB, Bhatt PA, Shah JS. Association between altered thyroid state and insulin resistance. J Pharmacol Pharmacother. 2012;3:156-60.
10. Spira D, et al. Association of thyroid function with insulin resistance: data from two population-based studies. Eur Thyroid J. 2022;11.
11. Mauvais-Jarvis F. Gender differences in glucose homeostasis and diabetes. Physiol Behav. 2018;187:20.
12. Ko GTC, Wai HPS, Tang JSF. Effects of age on plasma glucose levels in non-diabetic Hong Kong Chinese. Croat Med J. 2006;47:709.
13. Khan SR, et al. Thyroid function and cancer risk: The Rotterdam Study. J Clin Endocrinol Metab. 2016;101:5030-6.
14. Kim BJ, et al. Relationship between serum free T4 (FT4) levels and metabolic syndrome (MS) and its components in healthy euthyroid subjects. Clin Endocrinol (Oxf). 2009;70:152-60.
15. Sawicka-Gutaj N, et al. Influence of cigarette smoking on thyroid gland—an update. Endokrynol Pol. 2014;65:54-62.
16. Wang D, et al. Smoking causes the disorder of glucose metabolism under different levels of blood pressure in male occupational population. J Clin Hypertens. 2022;24:1276-84.
17. Medici M, Korevaar TIM, Visser WE, Visser TJ, Peeters RP. Thyroid function in pregnancy: what is normal? Clin Chem. 2015;61:704-13.
18. Mitchell K, Mukhopadhyay B. Drug-induced falsely low A1C: report of a case series from a diabetes clinic. Clin Diabetes. 2018;36:80.
19. Blendea MC, Thompson MJ, Malkani S. Diabetes and chronic liver disease: etiology and pitfalls in monitoring. Clin Diabetes. 2010;28:139-44.
20. Sathi P, Kalyan S, Hitchcock CL, Pudek M, Prior JC. Progesterone therapy increases free thyroxine levels—data from a randomized placebo-controlled 12-week hot flush trial. Clin Endocrinol (Oxf). 2013;79:282-7.

Correlation between serum ferritin level and haemoglobin concentration among potential blood donors in Hong Kong: A retrospective cohort study

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Abstract

Objectives: We screened for iron deficiency and anaemia among potential blood donors in Hong Kong, identified the biomarkers for iron monitoring and assessed the correlation between serum ferritin level and haemoglobin concentration. We, after all, intended to investigate the necessity and feasibility of serum ferritin testing in donation policy to monitor iron deficiency and anaemia in blood donors, ensuring sufficient and safe blood supply.

Materials & Methods: We conducted a retrospective cohort study based on data from a private laboratory's Laboratory Information System. Our study population was potential blood donors, healthy individuals aged between 16 and 65. In subject groups, we compared serum ferritin level and haemoglobin concentration, determined the prevalence of iron deficiency and anaemia, and illustrated the correlation between these biomarkers.

Results: Our results present a positive correlation between serum ferritin level and haemoglobin concentration. Females of reproductive age had the lowest mean serum ferritin level (16.81 ug/L) and haemoglobin concentration (11.01 g/dL) and the highest prevalence of iron deficiency (49.69%) and anaemia (62.63%). Our results also reinforce the current use of our cut-off values adopted by the private laboratory, accommodating the local population.

Conclusion: This study provides insight into the need for iron monitoring in blood donation services. The high prevalence of iron deficiency and anaemia raises public health concerns. We suggest including a serum ferritin check as part of the donation criteria and a potential solution to low and high deferral rates.

Keywords: *ferritin, haemoglobin, iron deficiency, anaemia, iron monitoring.*

Introduction

According to the Hong Kong Red Cross Blood Transfusion Service, only 2.34% of the eligible local population donated blood in 2020; 53% were men, and 47% were women (Hong Kong Red Cross, 2020). The service's low donation rate and high deferral rate remain two challenges. Low haemoglobin has been recognised as the main reason for donor deferral. Female donors are reportedly at higher risk than male donors (Browne *et al.*, 2019; Shi *et al.*, 2014).

We are interested in the prevalence of iron deficiency, anaemia and iron deficiency anaemia as reflected by either low serum ferritin, low haemoglobin or both. Ferritin is a specific biomarker for iron storage. Haemoglobin is a biomarker screening for blood diseases, beneficial for anaemia, and precisely one of our focuses. According to WHO guidelines, a serum ferritin level of <=15 ug/L is iron deficiency in healthy adolescents, adults and older persons (WHO, 2020). In 2019, the WHO reported that the global anaemia prevalence for females of reproductive age was 29.9% and 15.5% in China (WHO, 2023). While haemoglobin concentrations of <=12 g/dL in women and <=13 g/dL in men are anaemia (WHO, 2011). More importantly, iron deficiency is the primary contributor to anaemia. Common causes include but are not limited to poor iron absorption, heavy blood loss, hefty menstrual bleeding in women, and excess iron requirements in growing children and during pregnancy (WHO, 2020; Wu *et al.*, 2020).

We focus on the correlation between serum ferritin level and haemoglobin concentration. Most previous studies focused on the prevalence of anaemia in different population groups, different factors that contribute to anaemia, and diagnosis procedures and management of different types of anaemia (Bouri & Martin, 2018; Lin, 2021; Wu *et al.*, 2020). Limited studies discussed the relationship between haemoglobin, red blood cell (RBC) indices, serum ferritin and other iron biomarkers.

Besides, we question whether depleted iron storage can be identified with low serum ferritin before developing anaemia. Recent studies emphasised the importance of iron monitoring among blood donors (Spencer *et al.*, 2019; Sweegers *et al.*, 2020). Anaemia can be a temporary problem in frequent donors. Measuring serum ferritin level and haemoglobin concentration can be additional safety checks to avoid unnoticed iron depletion. This eventually helps to decrease low haemoglobin deferral, boosting donor return and improving blood transfusion service and the health of blood donors (Sweegers *et al.*, 2020).

Our objectives are to (1) determine the prevalence of iron deficiency and anaemia among potential blood donors in Hong Kong, (2) identify the biomarkers for screening iron deficiency and anaemia, and (3) further discuss the necessity for iron monitoring. We hypothesise that a positive correlation exists between serum ferritin level and haemoglobin concentration, and it is a positive biomarker for blood donors.

Materials & Methods

This retrospective cohort study was based on data collected from the private laboratory's Laboratory Information System (LIS) from July 2013 to July 2020. All patient data were anonymised; only patient demographics (gender and age), serum ferritin level and haemoglobin concentration were included. Our study population was potential blood donors in Hong Kong. A total of 834 healthy individuals aged between 16 and 65 with serum ferritin level and haemoglobin concentration results proceeded to statistical analysis. Software IBM SPSS Statistics version 26.0 (SPSS, Inc.) was used. Patients were subsequently categorised into three groups: males, females of reproductive age (aged 16-49) and postmenopausal females (aged 50-65).

Blood samples were collected, with complete

laboratory examinations in the private laboratory. Serum ferritin level was measured using chemiluminescent microparticle immunoassay (CMIA) by the Alinity system (Abbott, Illinois, USA). Haemoglobin concentration was measured in complete blood count (CBC) through automated haematology analysis by the Alinity system (Abbott, Illinois, USA). Reference intervals of serum ferritin levels adopted by the private laboratory were 21.8-274.0 ug/L in males and 4.6-204.0 ug/L in females. At the same time, haemoglobin concentration reference intervals were 13.3-17.5 g/dL in males and 11.5-15.2 g/dL in females.

Serum ferritin level and haemoglobin concentration were biomarkers for screening iron deficiency and anaemia. We first presented laboratory results of serum ferritin level and haemoglobin concentration as mean ± standard deviation (SD) and median. We then assessed the prevalence of iron deficiency and anaemia in males and females in frequency and percentage. Accommodating the local population, ferritin <=10 ug/L was considered an iron deficiency. While haemoglobin <=13 g/dL in males and <=11.5 g/dL in females signified anaemia. Iron deficiency anaemia describes patients with both iron deficiency and anaemia.

After that, we performed linear regression analysis with ANOVA to study the associations between serum ferritin level and haemoglobin concentration. This explained the effect of serum ferritin levels on haemoglobin concentration. To study the importance of the linear regression model, the null hypothesis H0 and alternative hypothesis H1 were listed below:

H0: There was no effect of serum ferritin level on haemoglobin concentration

H1: There was an effect of serum ferritin level on haemoglobin concentration

A p-value <0.05 was considered statistically significant, and there was evidence to reject the null hypothesis H0. The alternative hypothesis H1 was then accepted. A p-value >0.05 was considered not statistically significant, and there was evidence to retain the H0. We looked for significant associations between serum ferritin level and haemoglobin concentration.

Results

Serum ferritin level and haemoglobin concentration were shown in Table 1 as mean ± SD and Figure 1 as median. Of all potential blood donors in Hong Kong, male participants had the highest mean serum ferritin level (239.35 ± 142.99 ug/L) and mean haemoglobin concentration (13.71 ± 1.83 g/dL). They were the subject group most eligible to donate blood. Nevertheless, female participants aged 16-49 had the lowest mean serum ferritin level (16.81 ± 15.27 ug/L) and mean haemoglobin concentration (11.01 ± 1.75 g/dL).

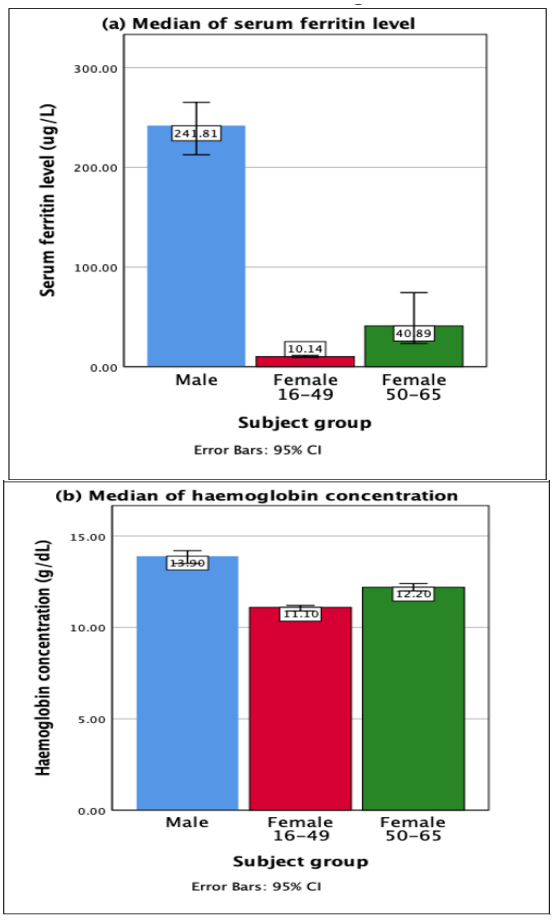

Figure 1. The median of (a) serum ferritin level and (b) haemoglobin concentration.

Besides, we compared the prevalence of iron deficiency and anaemia referring to cut-off

values suggested by WHO (ferritin <=15 ug/L, Hb <=13 g/dL in male, Hb <=12 g/dL in female) and used by the private laboratory (ferritin <=10 ug/L, Hb <=13 g/dL in male, Hb <=11.5 g/dL in female) in Table 2.

	Male (n = 241)	Female aged 16-49 (n = 487)	Female aged 50-65 (n= 106)	Total (n = 834)
Parameters	Mean ± SD			
Ferritin (ug/L)	239.35 ± 142.99	16.81 ± 15.27	80.97 ± 86.16	89.27 ± 128.68
Haemoglobin (g/dL)	13.71 ± 1.83	11.01 ± 1.75	12.06 ± 1.72	11.92 ± 2.13

Table 1. The mean of serum ferritin level and haemoglobin concentration

Our results reinforce the appropriate use of cut-off value established by the private laboratory in the local population (Hong Kong Red Cross, 2020). The total prevalence of iron deficiency dropped from 42.69% to 33.57%, anaemia from 56.00% to 50.24%, and iron deficiency anaemia from 36.81% to 29.26%. More potential blood donors were able to fulfil the donation criteria. Unsurprisingly, iron deficiency and anaemia were most common in females aged 16-49. Among 487 of them, 242 (49.69%), 305 (62.63%) and 215 (44.15%) had iron deficiency, anaemia and iron deficiency anaemia, respectively. Roughly half of them were rejected or deferred from blood donation.

In addition, we studied the necessity to include serum ferritin testing in iron monitoring. Figure 2 presents the distribution graphs of participants. A positive correlation (r = 0.524) between serum ferritin level and haemoglobin concentration was reported. R2 (0.275) measured that 27.5% of the variance in haemoglobin concentration was due to serum ferritin level. The remaining 72.5% could be explained by individual variation and other factors(Kumari & Yadav, 2018). The p-value (0.000) was <0.05.

	Iron deficiency		Anaemia		Iron deficiency anaemia	
Cut-off (WHO)	Ferritin <= 15 ug/L		Hb <=13 g/dL (male) Hb <= 12 g/dL (female)		Ferritin <=15 ug/L and Hb <=13 g/dL (male) or Hb <= 12 g/dL (female)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Total (n = 834)	356/834	42.69%	467/834	56.00%	307/834	36.81%
Cut-off (Private laboratory)	Ferritin <= 10 ug/L		Hb <= 13 g/dL (male) Hb <= 11.5 g/dL (female)		Ferritin <= 10 ug/L and Hb <= 13 g/dL (male) or Hb <= 11.5 g/dL (female)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Male (n = 241)	13/241	5.39%	78/241	32.37%	12/241	4.98%
Female aged 16-49 (n = 487)	242/487	**49.69%**	305/487	**62.63%**	215/487	**44.15%**
Female aged 50-65 (n = 106)	25/106	23.58%	36/106	33.96%	17/106	16.04%
Total (n = 834)	280/834	**33.57%**	419/834	**50.24%**	244/834	**29.26%**

Table 2. Prevalence of iron deficiency and anaemia according to subject groups with cut-off values suggested by WHO and adopted by the private laboratory.

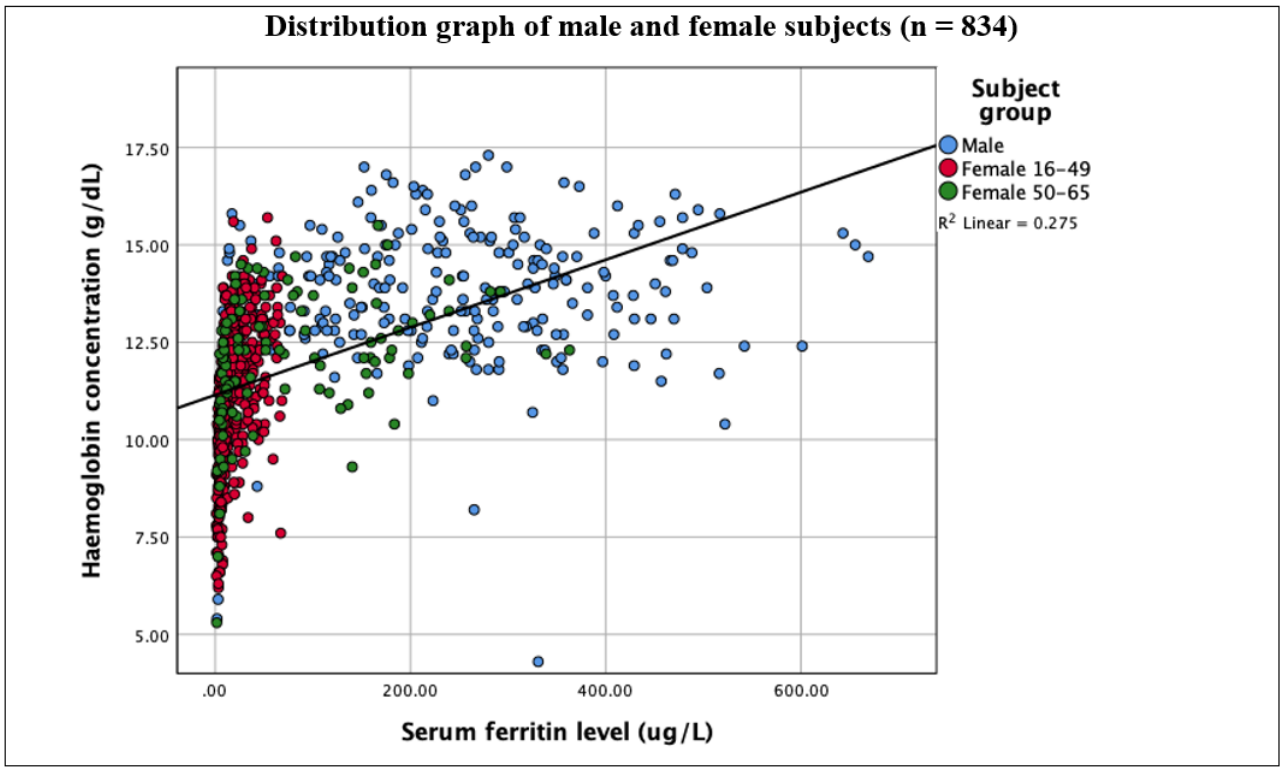

Figure 2. Distribution of male and female subjects (n = 834)

Three subject groups also noticed positive correlations (P <0.05). Therefore, it was statistically significant to accept the alternative hypothesis H1, which there was an effect of serum ferritin level on haemoglobin concentration. Low serum ferritin levels reflected low iron storage and low haemoglobin concentration.

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Discussion

Our representative (n = 834) study presented the association of serum ferritin level with haemoglobin concentration and the prevalence of iron deficiency and anaemia among potential blood donors in Hong Kong (Table 2). We indicate that serum ferritin level positively correlates with haemoglobin concentration (Figure 2) and suggest that serum ferritin level could be included with haemoglobin concentration as biomarkers in blood donation services for screening iron deficiency and anaemia.

As briefly introduced, few studies explained the correlation between serum ferritin level and haemoglobin concentration. Nevertheless, they were carried out in Western countries, not Hong Kong or other Asian countries. Our results help to fill the gap in our understanding. We accepted the hypothesis that serum ferritin level affects haemoglobin concentration. Lower serum ferritin levels lead to lower haemoglobin concentration, supporting the idea that iron deficiency causes anaemia.

Previous studies found a positive correlation between serum ferritin level and haemoglobin concentration (Guo *et al.*, 2013; Timmer *et al.*, 2020). Besides, more studies showed low serum ferritin levels and low haemoglobin concentrations in iron deficiency anaemia (Bouri & Martin, 2018; Jansen, 2019). However, factors other than iron deficiency could contribute to low haemoglobin, brief infection or inflammation, thalassemia diseases, body mass index (BMI), blood loss, dietary practice, smoking, etc. (Weiss *et al.*, 2019; WHO, 2011). Also, serum

ferritin level is associated with iron deficiency and other diseases, such as systemic sclerosis (Jiang et al., 2022) and multiple myeloma (Plano et al., 2023). Recent studies focused on the correlation between serum ferritin levels and outcomes in Covid-19 hospitalised patients (Ishikura et al., 2023; Shakaroun et al., 2023). These circumstances complicate the role of serum ferritin levels in iron screening.

We hope our findings help improve blood donation service and encourage more local people to become regular blood donors. Learning from practice in well-developed blood transfusion services, we discuss the feasibility of including serum ferritin level and haemoglobin concentration biomarkers in iron monitoring.

In practice, haemoglobin concentration is the only pre-donation test screening for iron deficiency and anaemia. Low haemoglobin has been identified as a critical cause of donor deferral. In 2017, the Hong Kong Red Cross reported that 22% of donors were temporarily deferred, primarily due to low haemoglobin and travel history (Hong Kong Red Cross, 2020). Deferring blood donors can lead to a blood supply shortage. Donors can also feel discouraged and have less intention to return for a donation, especially first-time donors (Yang, 2021). An important note is that haemoglobin concentration has limited sensitivity in detecting early stages of iron deficiency. On the other hand, serum ferritin levels reflect depleted iron storage more accurately and serve as a better biomarker than serum iron levels and haemoglobin concentrations (Sweegers *et al.*, 2020).

Internationally, there is no compulsory iron monitoring policy in blood transfusion services. In Switzerland, routine serum ferritin testing was first introduced in pre-donation screening in 2004 (O'Meara *et al.*, 2011). Such practice monitored and allowed effective management of iron deficiency in blood donors. Moreover, the prevalence of pre-donation anaemia was dropped, and the donor return rate was boosted with shorter donation intervals (O'Meara *et al.*, 2011). In Denmark, a recent study presented that routine serum ferritin testing and iron supplements helped reduce donor deferral because of low haemoglobin (Rigas *et al.*, 2019). Locally, the Hong Kong Red Cross recorded a significant drop in blood donation yet a high deferral rate (Hong Kong Red Cross, 2020). It is necessary to screen for underdiagnosed iron deficiency to optimise management in donors and reduce donor deferral.

In addition to iron monitoring, serum ferritin testing among blood donors enables appropriate adjustment of donation interval and further guides iron supplements. In the Netherlands, recent research focused on implementing ferritin-guided donation intervals. Blood banks assessed iron storage among donors by serum ferritin level. Those with low serum ferritin measurement were asked to extend their donation interval to 6-12 months. Blood donors were also enrolled in a ferritin-guided iron supplement programme (Karregat *et al.*, 2021; Sweegers *et al.*, 2020). This provides more time to restore iron, increase serum ferritin levels, and decrease low haemoglobin deferral (Sweegers *et al.*, 2020).

Our discussion is based on the most updated research papers. There are several limitations to our study. Participants were grouped by their gender and age. If more information is provided, we can more thoroughly characterise them by socioeconomic background, physical fitness, dietary habits, and smoking status, contributing to iron deficiency and anaemia. A retrospective cohort study design also makes it challenging to conduct a follow-up study. We recommend future studies, such as evaluating how serum ferritin measurement helps improve iron monitoring and how frequent blood donation affects iron storage in local blood donors. We are also interested in examining the feasibility of following policies in Western countries establishing ferritin-guided donation interval and iron supplement programmes in our local blood transfusion service.

Conclusion

This study overviews the correlation between serum ferritin level and haemoglobin concentration. Our results reinforce the reliability of previous findings. We agree that serum ferritin level and haemoglobin concentration cut-off values adopted by the private laboratory are more applicable than those recommended by WHO. We also present the prevalence of iron deficiency and anaemia among potential blood donors in Hong Kong, suggesting including serum ferritin level and haemoglobin concentration as biomarkers in iron monitoring. Lastly, we need further studies to discuss how serum ferritin measurement can be appropriately added to current donation criteria in blood transfusion services.

References

1. Bouri S, Martin J. Investigation of iron deficiency anaemia. Clin Med (Lond). 2018;18(3):242-4. doi:10.7861/clinmedicine.18-3-242.
2. Browne A, Fisher SA, Masconi K, Smith G, Doree C, Chung R, et al. Donor deferral due to low hemoglobin – An updated systematic review. Transfus Med Rev. 2019;34(1):10-22. doi:10.1016/j.tmrv.2019.10.002.
3. Guo X, Zhou D, An P, Wu Q, Wang H, Wu A, et al. Associations between serum hepcidin, ferritin and Hb concentrations and type 2 diabetes risks in a Han Chinese population. Br J Nutr. 2013;110(12):2180-5. doi:10.1017/S0007114513001827.
4. Hong Kong Red Cross. Hong Kong Red Cross Blood Transfusion Service [Internet]. 2020 [cited 2024 Sep 16]. Available from: https://www5.ha.org.hk/rcbts/.
5. Ishikura T, Nakano T, Kitano T, Tokuda T, Sumi-Akamaru H, Naka T. Serum ferritin level during hospitalisation is associated with brain fog after Covid-19. Sci Rep. 2023;13(1):13095. doi:10.1038/s41598-023-40011-0.
6. Jansen V. Diagnosis of anemia – A synoptic overview and practical approach. Transfus Apher Sci. 2019;58(4):375-85. doi:10.1016/j.transci.2019.06.012.
7. Jiang Y, Li X, Zhou W, Jin M, Li S, Lao Y, et al. Clinical significance of serum ferritin in patients with systemic sclerosis. J Clin Lab Anal. 2022;36(8):e24597. doi:10.1002/jcla.24597.
8. Karregat J, Sweegers MG, Quee FA, Weekamp HH, Swinkels DW, Novotny VMJ, et al. Ferritin-guided iron supplementation in whole blood donors: Optimal dosage, donor response, return and efficacy (FORTE) – A randomised controlled trial protocol. BMJ Open. 2022;12(3):e056316. doi:10.1136/bmjopen-2021-056316.
9. Kumari K, Yadav S. Linear regression analysis study. Curr Cardiol. 2018;4(1):33-6. doi:10.4103/jpcs.jpcs_8_18.
10. Lin SY. Management of anaemia in primary care. J Hong Kong Coll Fam Physicians. 2021;43:2. Available from: https://www.hkcfp.org.hk/Upload/HK_Practitioner/2021/hkp2021vol43Jun/update_article.html.
11. O'Meara A, Infanti L, Stebler C, Ruesch M, Sigle JP, Stern M, et al. The value of routine ferritin measurement in blood donors. Transfusion. 2011;51(10):2183-8. doi:10.1111/j.1537-2995.2011.03148.x.
12. Plano F, Gigliotta E, Corsale AM, Azgomi MS, Santonocito C, Ingrascì M, et al. Ferritin metabolism reflects multiple myeloma microenvironment and predicts patient outcome. Int J Mol Sci. 2023;24(10):8852. doi:10.3390/ijms24108852.
13. Rigas AS, Pedersen OB, Magnussen K, Erikstrup C, Ullum H. Iron deficiency among blood donors: Experience from the Danish Blood Donor Study and from the

Copenhagen ferritin monitoring scheme. Transfus Med. 2019;29(S1):23-7. doi:10.1111/tme.12477.

14. Shakaroun DA, Lazar MH, Horowitz JC, Jennings JH. Serum ferritin as a predictor of outcomes in hospitalised patients with Covid-19 pneumonia. J Intensive Care Med. 2023;38(1):21-6. doi:10.1177/08850666221113252.

15. Shi L, Wang J, Liu Z, Stevens L, Sadler A, Ness P, et al. Blood donor management in China. Transfus Med Hemother. 2014;41(4):273-82. https://doi.org/10.1159/000365425

16. Spencer BR, Bialkowski W, Creel DV, Cable RG, Kiss JE, Stone M, et al. Elevated risk for iron depletion in high-school age blood donors. Transfusion. 2019;59(5):1706-16. https://doi.org/10.1111/trf.15133

17. Sweegers MG, Zalpuri S, Quee FA, Huis in't Veld EMJ, Prinsze FJ, Hoogendijk EO, et al. Ferritin measurement in donors – Effectiveness of iron monitoring to diminish iron deficiency and low haemoglobin in whole blood donors (FIND 'EM): Study protocol for a stepped wedge cluster randomised trial. Trials. 2020;21:823. https://doi.org/10.1186/s13063-020-04648-w

18. Timmer TC, De Groot R, Rijnhart JJBM, Lakerveld J, Brug J, Perenboom CWM, et al. Dietary intake of heme iron is associated with ferritin and haemoglobin levels in Dutch blood donors: Results from Donor Insight. Haematologica. 2020;105(10):2400-6. https://doi.org/10.3324/haematol.2019.229450

19. Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. Blood. 2019;133(1):40-50. https://doi.org/10.1182/blood-2018-06-856500

20. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva: WHO; 2011. WHO/NMH/NHD/MNM/11.1.

21. WHO. WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. Geneva: WHO; 2020. ISBN 978-92-4-000012-4.

22. WHO. WHO global anaemia estimates, 2021 edition. The Global Health Observatory. Available from: https://www.who.int/data/gho/data/themes/topics/anaemia_in_women_and_children.

23. Wu Y, Ye H, Liu J, Ma Q, Yuan Y, Pang Q, et al. Prevalence of anemia and sociodemographic characteristics among pregnant and non-pregnant women in southwest China: A longitudinal observational study. BMC Pregnancy Childbirth. 2020;20:535. https://doi.org/10.1186/s12884-020-03222-1

24. Yang Y. Characteristics and causes of pre-donation deferrals at a Chinese blood center. Transfus Apher Sci. 2021;60(4):103153. https://doi.org/10.1016/j.transci.2021.103153

Recent prevalence of human papillomavirus (HPV) genotypes with cytological findings in Hong Kong: 2020-2021

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Abstract

Background: Human papillomavirus (HPV) is the primary cause of the development of cervical cytological abnormality. This research evaluates the prevalence rate and genotype distribution of human papillomavirus (HPV) to support and assess the effectiveness of the current vaccination program. We aim to investigate the recent pattern of HPV genotypes, which are closely correlated to cervical abnormality in Hong Kong.

Objectives: To investigate the association between cytological findings, age, sex, and HPV genotypes found in the Hong Kong population to detect the recent prevalence and study the changes in the pattern of HPV genotypes.

Materials and Methods: From January 2020 to December 2021, a private laboratory collected 2530 random ThinPrep specimens from females aged between 17 and 83. Data analysis was done using Microsoft Excel and SPSS.

Results: The overall prevalence of HPV was 54.6% (N=1381/2530) from 2020 to 2021. The highest infection rate was in the age group <=30. The three most commonly found HR-HPV subtypes in HPV-positive cases were HR-52 (10%), HR-53 (5.9%), and HR-58 (5.8%) in 2020. However, the three most commonly found HR-HPV subtypes in HPV-positive cases were HR-81 (10.9%), HR-40 (5.4%), and HR-47 (6.3%) in 2021. In contrast, the most common LR-HPV subtype between 2020 and 2021 was LR-43 (4.9%).

Conclusion: This study revealed the increasing HPV infection rate and genotype distribution among women aged 30 years or below, especially for HR-52 as the dominant subtype with a higher risk of causing cervical abnormality in recent years in Hong Kong, which could serve as vital and statistical foundation for considering HPV vaccination and preventative strategies to minimize the risk for cervical pre-invasive disease. A significant decline of 30.1% HPV positive rate in the ASC-US population, a success with the diminishing prevalence rate of HR-18 and HR-31, and a positive impact of the HPV vaccine is the outcome.

Keywords: *human papillomavirus, age, cytology, vaccination, prevalence rate, HPV genotype*

Introduction

Scientific Background

Human papillomavirus (HPV) stands as the pivotal factor in the development of cervical neoplasia. This correlation is particularly strong with high risk (HR-HPV) and low risk (LR-HPV) genotypes[1], which are known to lead to the development of cervical intraepithelial neoplasia (CIN, grade 3) or cervix cancer (CIN, > grade 3)[2]. The presence of HPV in the infected host is a consistent marker of cervical cancerous cells due to the cervix embedding HPV-DNA. HPV gains entry into the basal cell layer of the epithelium in the event of cervical epithelium trauma. The initial infection often occurs at the site between the ecto- and endo-cervix. The "transformation zone" is a term used to describe the frequent occurrence of cervical cell neoplasia in the cervix malignancy.

An epidemiological study in recent years has indicated the prevalence of HR-HPV, which correlates to cytological abnormality. Five significant cytology abnormalities, Atypia ASC-US, AGC, Atypia ASC-H, LSIL, and HSIL, are commonly used for cytology screening reporting by the Bethesda system (2014)[3,4]. Cervical cytological abnormalities are classified as follows: Atypical Squamous Cell of Undetermined Significance (ASCUS), Atypical Squamous Cells include High-grade squamous intraepithelial lesion (ASC-H), Low-grade Squamous Intraepithelial Lesion (LSIL), Atypical Glandular Cell (AGC), High-grade Squamous Intraepithelial Lesion (HSIL), and Squamous Cell Carcinoma (SSC).

The global prevalence of HPV infection, including normal cervical cytology, is estimated at 16.93% in 2022 based on systematic reviews and meta-analysis performed by the Catalan Institute of Oncology (ICO)/ IARC HPV Information Centre[5]. With a comparison, this estimation of global HPV prevalence in 2022 is higher than that of the majority, 11.7% in 2010[6]. An exceptionally high prevalence of HPV infection resulted in Oceania (21.8%) and Africa (21.1%), followed by Asia (9.4%). For gynecologically healthy and unhealthy women who were HPV carriers in 2011, Asia and Africa had the highest prevalence of 45.5 and 29.6%, respectively[7]. In almost all European countries, the HPV prevalence was low (<30%), as in Western Europe (3.7%)[8]. There have been many studies worldwide on the epidemiology of HPV infection and oncogenic properties due to different HPV genotypes.

In a recent study, 55% of cervical cancers belong to high-risk HPVs (HR-HPVs)[9]. The most common types of HR-HPV worldwide are in descending order HPV-16, HPV-18, HPV-59, HPV-45, HPV-31, HPV-33, HPV-52, HPV-58, HPV-35, HPV-39, HPV-51, HPV-56 and HPV-53. High-risk types of HPV are also distributed unevenly by region, with HPV-16, HPV-18, HPV-59, and HPV-73 having a distribution rate of around 32.1%, 5.8%, 5.2% and 3.5%, separately in the world[10]. For Asian women infected with HPV-16, the rate of invasive cervical abnormality and normal cervical cytology are 60.5%[11] and 2.5%[12]. In Figure 1A, the features and hallmarks for HPV genetic expression include the construction of viral capsomers such as L1 and L2 proteins forming icosahedral protein shells, for the recognition site of origin of replication such as E1 and E2 proteins, which is for viral gene transcription. Negative cell cycle regulators, such as E6 and E7 proteins (shown in Figure 1B), facilitate stable viral genomes and protein maintenance and start a persistent infection[13,14].

Publication[15] stated that the reason for HR-HPV infection (e.g., HPV-16) is related to the precise loci of HPV-16. HPV-16 expresses histone post-translational modifications (PTMs) associated with the enhancer and promoters (p97 and p670) at HPV nucleosomes during the progression of in-vitro neoplastic progression to develop cervical squamous cell carcinoma (SCC)[16].

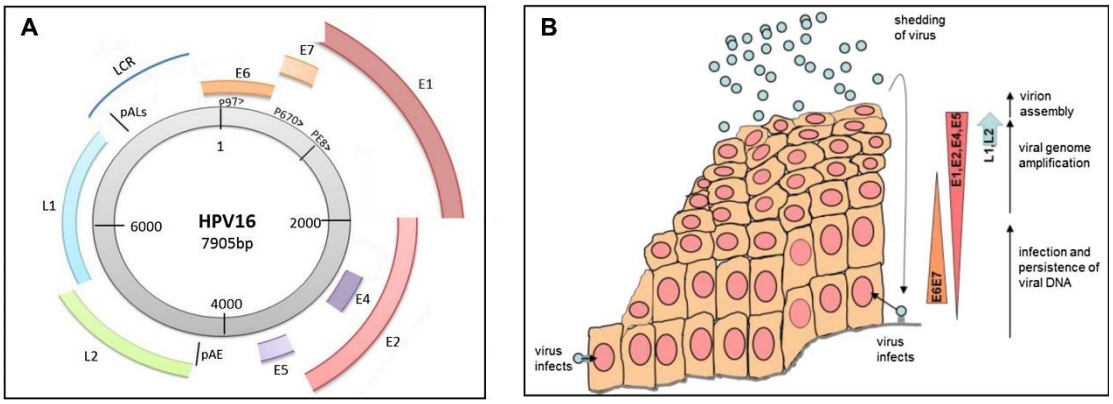

Figure 1A. Summary of HPV-16 induced cervical carcinoma with major regulatory or capsid proteins, oncogenes, and replication factors involved in establishing cervical cancer adapted from hallmarks of cancer; Figure 1B. The invasive HPV-16 life cycle of replication in a differentiating epithelium. Adopted from the publication[14].

A recent study in China indicated the typical pattern of HPV infections caused by HR-HPV (71.8%). The single genotype of HPV accounted for 13.7%. The most common HR-HPV genotype was HPV-16 (4.3%), followed by HPV-52 (3.5%) and HPV-58 (2.0%)[17]. HPV infection has reached a considerable proportion worldwide, particularly among women, in whom it is the primary cause of cancer[18], therefore making HPV to be the highest priority of global public health issues. Moreover, prevalence and type distribution are heterogeneous, especially in Hong Kong. The prevalence of HPV positivity is 39.4%3. The age-specific HPV prevalence varies and shows a pronounced peak in young (31-40 years, 34.5%) and advanced age (≧51 years, 47.1%) women populations19. And, the publication19 revealed that HPV-66 (8.3%, n=108) was the most prevalent genotype, followed by HPV-16 (6.5%), HPV-53 (5.6%), HPV-52 (4.6%), HPV-56 (4.6%) and HPV-73 (4.6%) in Hong Kong.

Nowadays, vaccines now available in the market include bivalent against HPV-16 and HPV-18 (CervarixTM, licensed in 2009), quadrivalent against HPV-6, HPV-11, HPV-16 and HPV-18 (Gardasil, approved in 2006), and 9-valent against the high risk of HPV-16, 18, 31, 33, 45, 52, 58 and low risk of HPV-6, 11 (Approved by the US Food and Drug Administration (FDA) in 2014). Currently, the HPV vaccination program significantly minimises the prevalence of HPV infection as a public health problem in Hong Kong.

Materials and Methods

Subject data

ThinPrep samples were collected from a private laboratory to perform cytological slides for pathologists to review and report any cytological abnormality with the HPV DNA test for different HPV genotypes. Pap stain is commonly used for slide examination by the IAC screener if the cases are negative or the pathologist if the cases are positive.

A total of 2530 samples' (Hong Kong women aged between 17-83 years old) retrospective data, including patients' age, cervical cytological results, and HPV genotype results, were collected from January 2020 to December 2021. The laboratory results were examined by DNA extraction with the high pure viral nucleic acid kit (Roche, USA) and detection by SNIPERTM high-throughput DNA microarray HPV genotyping system to obtain qualitative results of HPV DNA

genotypes.

One Hong Kong private laboratory generates the Excel data format through the Laboratory Information System (LIS). Sample data used in this study were unaccompanied, including identifying patient information. These data were used to analyse the relationship between cervical cytological findings and HPV genotype(s) within them - no effect to the patient's diagnosis and treatment.

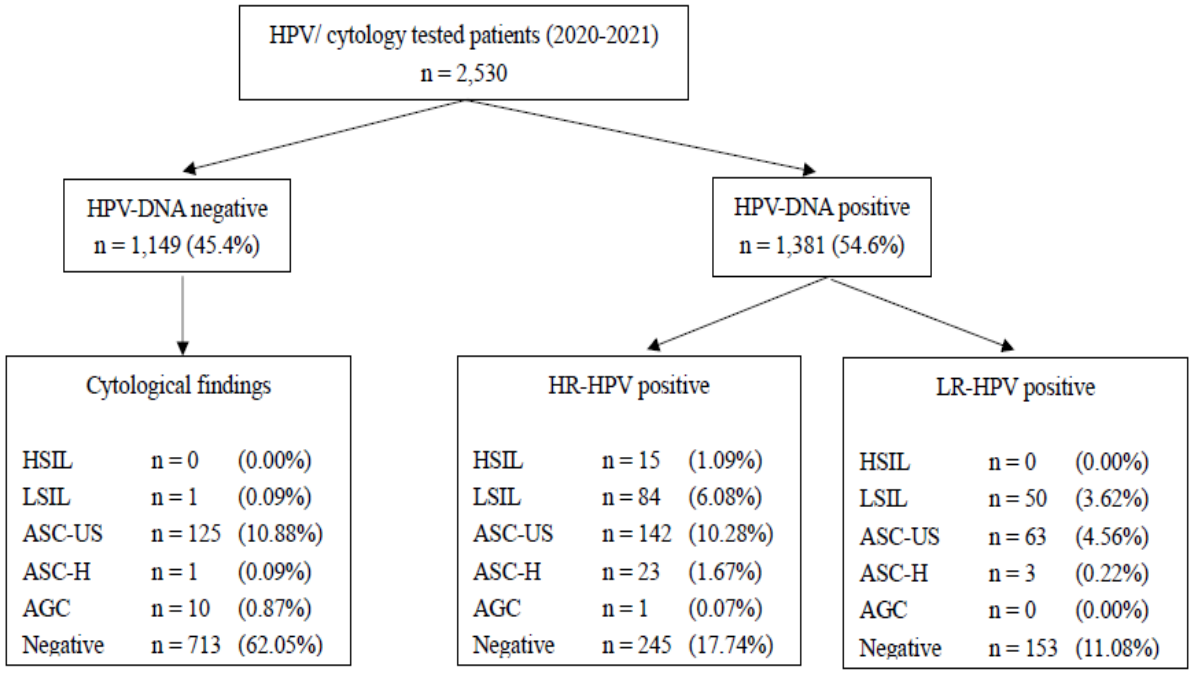

Figure 2. Sampling Model – A flow diagram of patient selection from a randomized sampling pool to a study group. [Remark: HSIL = high-grade squamous intraepithelial neoplasia, LSIL = low-grade squamous intraepithelial neoplasia, ASC-US = atypical squamous cells of undetermined significance, ASC-H = atypical squamous cells-cannot exclude high-grade squamous intraepithelial neoplasia, AGC = atypical glandular cell, Negative = negative for intraepithelial lesions or malignancy]

Data analysis

Retrospective datasets analysis of HPV genotypes, cervical cytology and patients' age will be performed by using a statistical program (e.g. SPSS®) to perform Fisher's exact test and Pearson Chi-square test for analysis of statistical significance (P value <0.05 was considered significant), and Microsoft Excel® for data set integration. The 95% confidence level and confidence intervals were used. Finally, the analysed results will be generated and presented in graphs or tables whenever required.

Results

All collected samples (n=2530) from 2020 to 2021 were utilised for cytological study and HPV genotyping. In Figure 3, 54.6% (1381/2530) of all cases were determined to be infected with any HPV genotype.

HPV Overall Prevalence

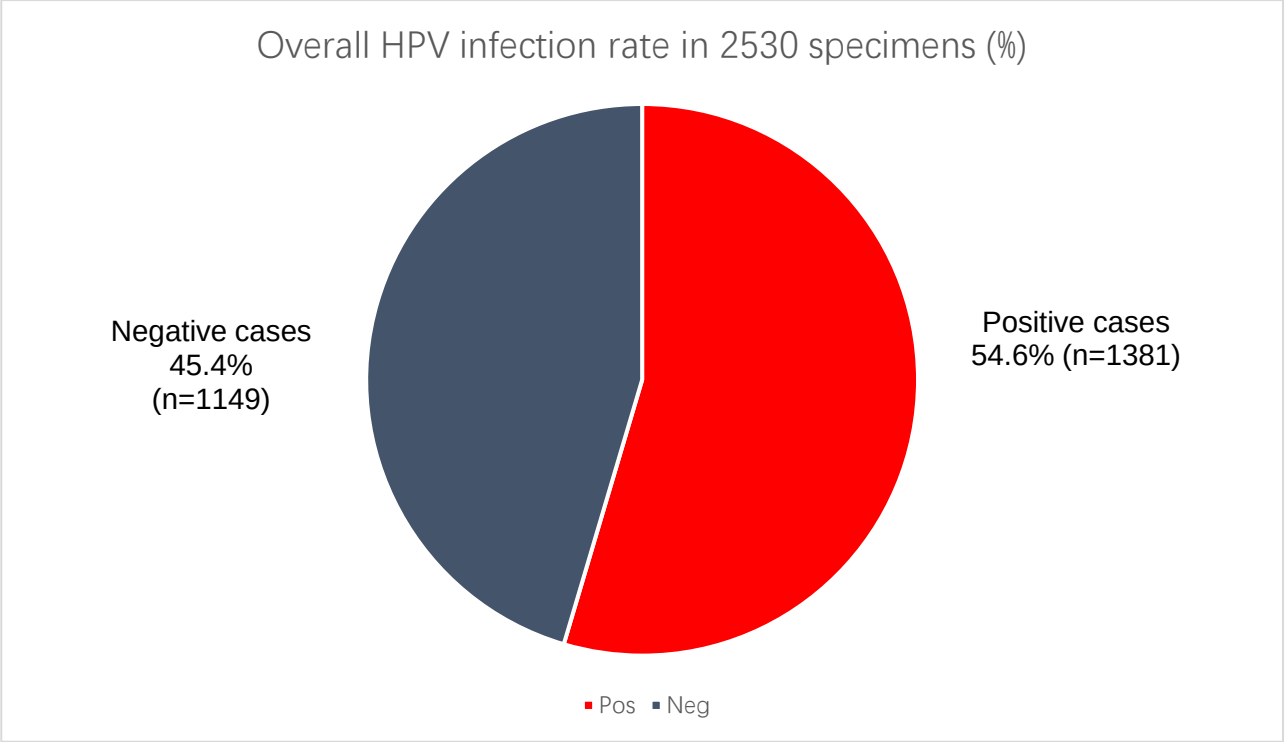

Figure 3. Overall HPV infection rate in 2530 specimens (%).

Table 1. Data showing the mean, standard deviation, and variance for the resulted prevalence rate of HR-HPV and LR-HPV from 2020 to 2021.

2020	N (no. of population)	Prevalence rate % (95% CI)	Mean	SD	Variance
LR-HPV	269	10.73 (12.28 - 26.72)	17.83	5.717	26.14446
HR-HPV	371	26.98 (29.82 - 44.83)	35.96	7.23	41.81906

2021	N (no. of population)	Prevalence rate % (95% CI)	Mean	SD	Variance
LR-HPV	342	16.98 (6.6 - 32.71)	17.98	9.36	70.08322
HR-HPV	399	32.85 (19.1 - 49.06)	32.87	13.34	142.27906

The prevalence of HR-HPV genotypes in 2020-2021 is shown in Figure 4. A total of 17 HPV genotypes were identified. The most frequently detected HR-HPV genotypes in descending order were HPV-52 (Mean = 10.45%), HPV-53 (Mean = 5.65%), HPV-58 (Mean = 6.05%), HPV-66 (Mean = 5.55%), HPV-16 (Mean = 3.95%) and HPV-68 (Mean = 5.1%).

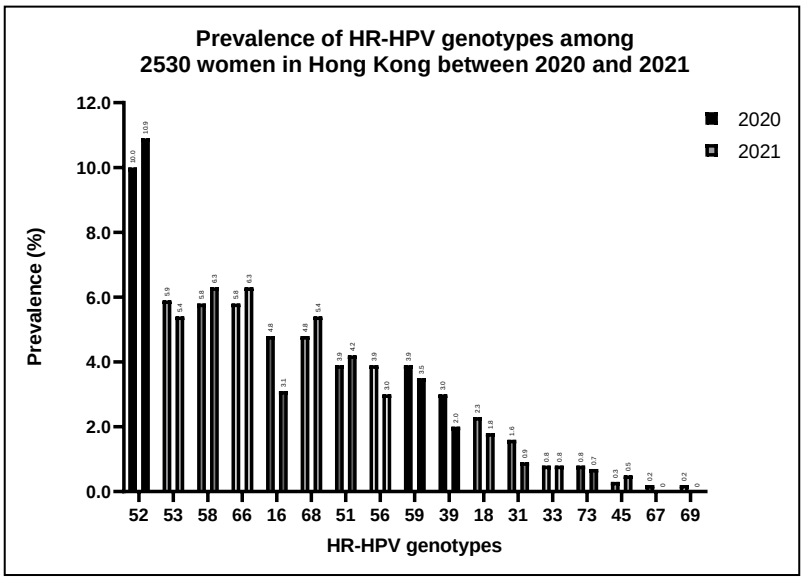

Figure 4. Prevalence of HPV genotypes among 2530 women in Hong Kong. HR-HPV includes HPV-52, 53, 58, 66, 16, 68, 51, 56, 59, 39, 18, 31, 73, 45, 67, and 69. The HR-HPV genotypes are arranged according to the prevalence in decreasing order. [Remark: Women may be counted more than once for multiple infections.]

The prevalence of LR-HPV genotypes in 2020-2021 is shown in Figure 4.1. A total of 23 HPV genotypes were identified. The most frequently detected LR-HPV genotypes in descending order were HPV-44 (Mean = 4.95%), HPV-43 (Mean = 4.85%), HPV-61 (Mean = 4.25%), HPV-54 (Mean = 4.1%), HPV-40 (Mean = 3.95%) and HPV-55 (Mean = 3.95%).

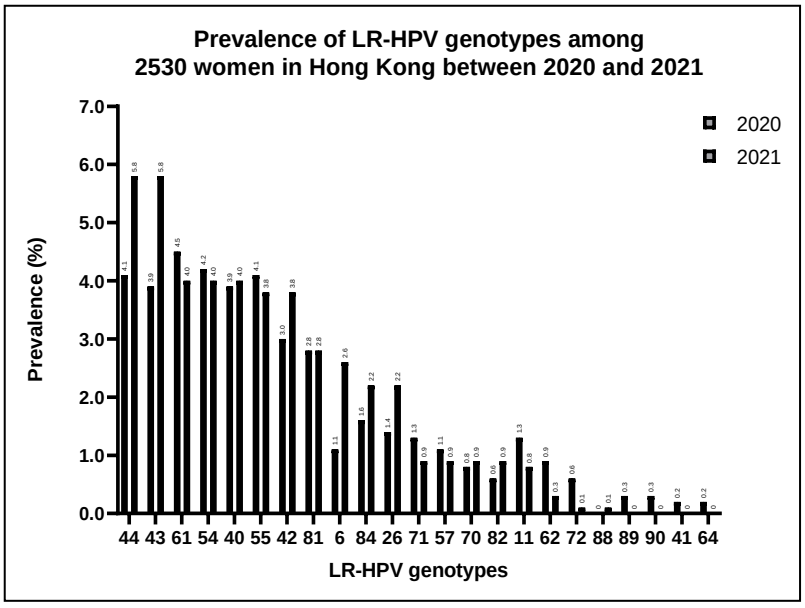

Figure 4.1. Prevalence of HPV genotypes among 2530 women in Hong Kong. LR-HPV includes HPV-44, 43, 61, 54, 40, 55, 42, 81, 6, 84, 26, 71, 57, 70, 82, 11, 62, 72, 88, 89, 90, 41, and 64. The LR-HPV genotypes are arranged according to the prevalence. [Remark: Women may be counted more than once for multiple infections.]

High-risk Genotypes	Number of cases (2020, n=718)	Number of cases (2021, n=1812)	Trend (e.g. increasing/ decreasing %)
68	31	40	↑ 29
58	37	47	↑ 27
52	64	81	↑ 26.6

Table 2. HR-HPV 68, 58, and 52 with an obvious increasing trend were 29%, 27%, and 26.6% by a comparison between 2020 and 2021.

Low-risk Genotypes	Number of cases (2020, n=718)	Number of cases (2021, n=1812)	Trend (e.g. increasing/ decreasing %)
6	7	19	↑ 71
26	9	16	↑ 77.8
82	4	7	↑ 75

Table 3. LR-HPV 6, 26, and 82 with an obvious increasing trend were 71%, 77.8%, and 75% by a comparison between 2020 and 2021.

Cytological Results vs HPV Infection

Figure 5 shows that the detected HR-HPV infection correlated with cervical findings (e.g. HSIL/ LSIL, ASC-US/ ASC-H) which is higher than that in detected LR-HPV.

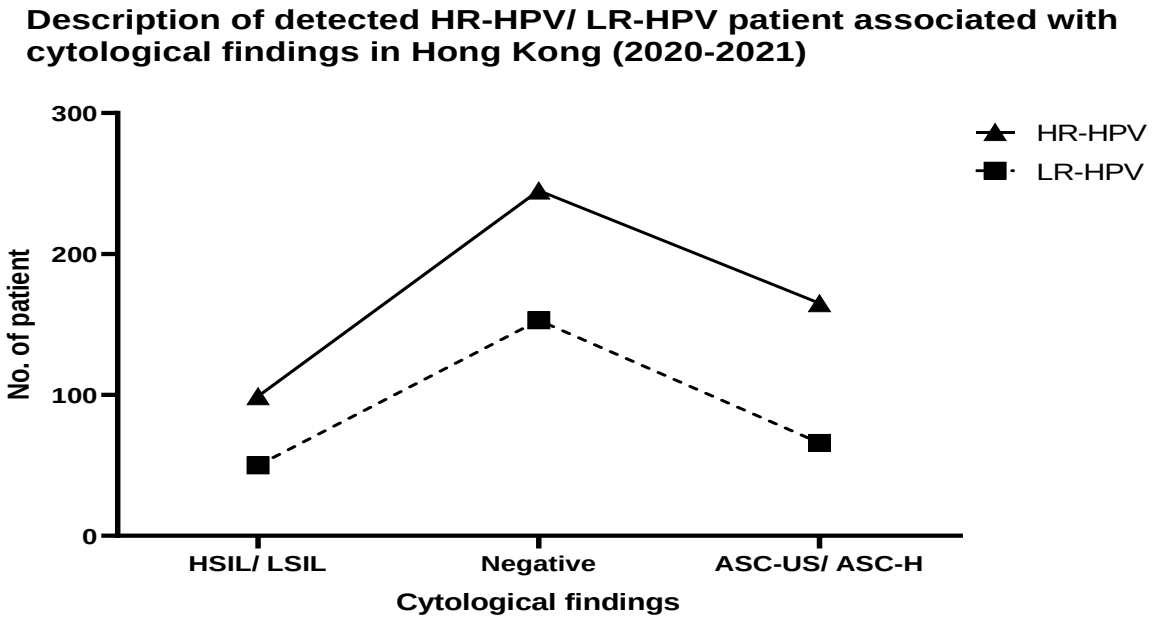

Figure 5. Description of detected HR-HPV/ LR-HPV patients associated with cytological findings except AGC cases (HSIL/LSIL with HR-HPV/ LR-HPV = 13%/ 6%, n=99/ 50; ASC-US/ ASC-H with HR-HPV/ LR-HPV = 21%/ 8%, n=165/ 66) in Hong Kong between 2020 and 2021 (n = 778).

However, Figure 5.1 shows that the HPV infection rate was more than 30% in most cytological abnormalities except atypical glandular cells (AGC). The highest HPV prevalence with abnormal cytological results is in low-grade squamous intraepithelial lesions (LGSIL). LGSIL was a cervical lesion of the pre-invasive stage, which is more severe than a high grade of atypical squamous cell intraepithelial lesion (ASC-H), atypical squamous cell of undetermined significance (ASC-US), and AGC.

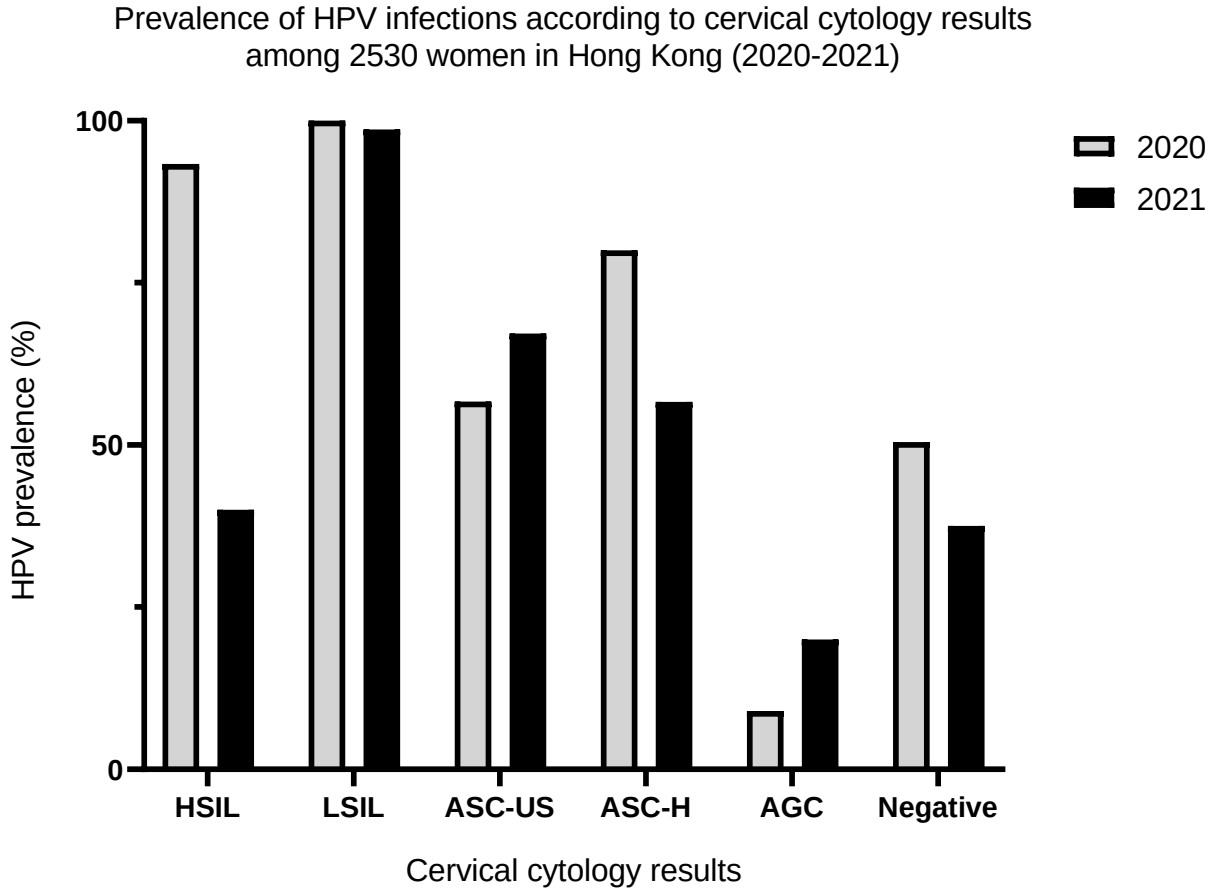

Figure 5.1 Prevalence of HPV infections according to cervical cytology results among 2530 women in Hong Kong. HPV positive indicates any HPV genotype infection. The HPV-positive rate of women with LSIL is higher than the others. AGC is the lowest rate of HPV-positive infection.

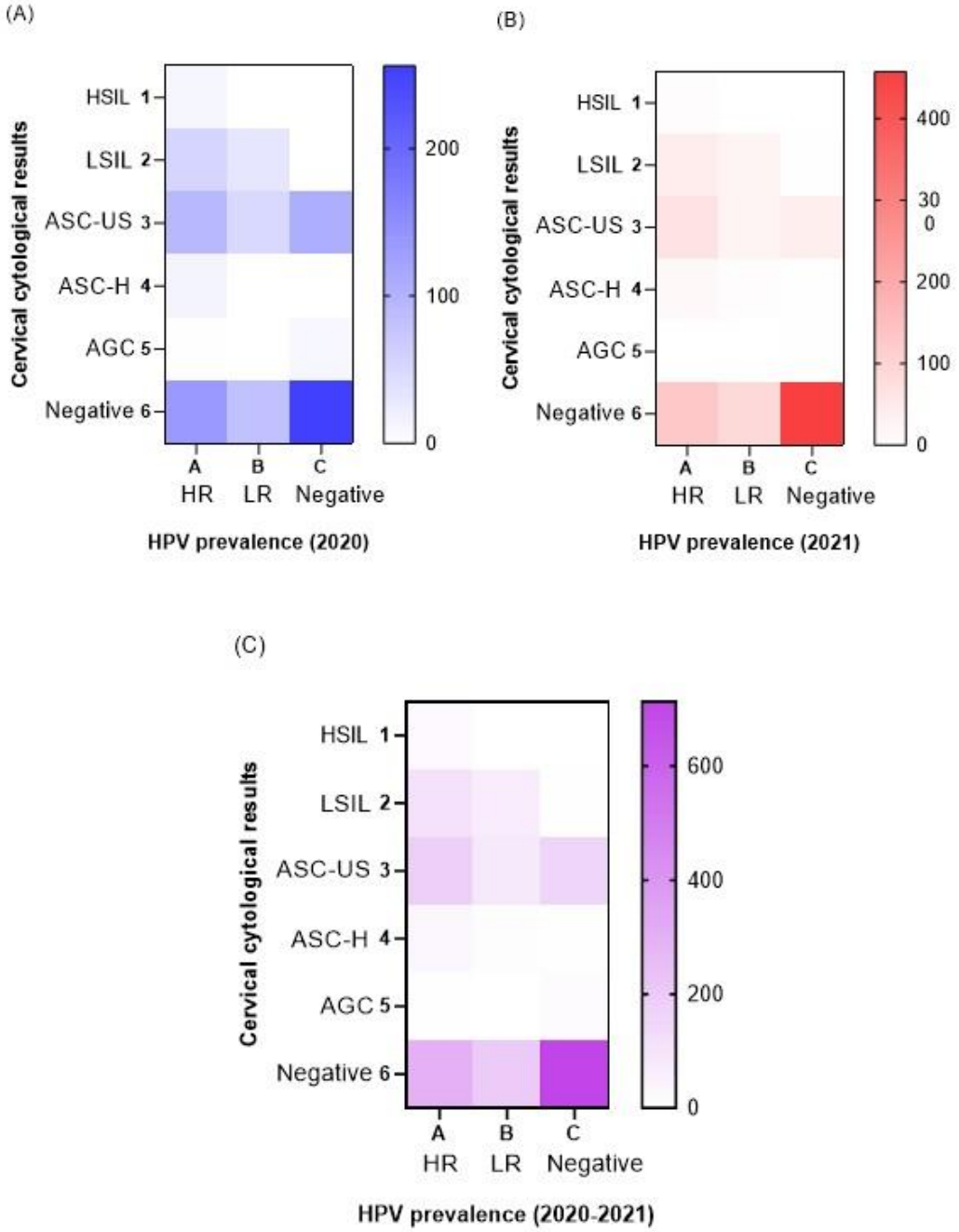

Figure 5.2. Heat maps showing the prevalence of HPV infections according to cervical cytology results among 718, 1812, and 2530 women in Hong Kong (2020) shown in Figure 5.2.A., (2021) shown in Figure 5.2.B., and (summary of 2020-2021) shown in Figure 5.2.C. separately. HPV positive indicates any HR-HPV or LR-HPV genotype infection. In 2020-2021, the HPV-positive rate of women with ASC-US (12.6%, n = 320/2530) was higher than the others. HSIL is the only HPV-positive infection by HR-HPV.

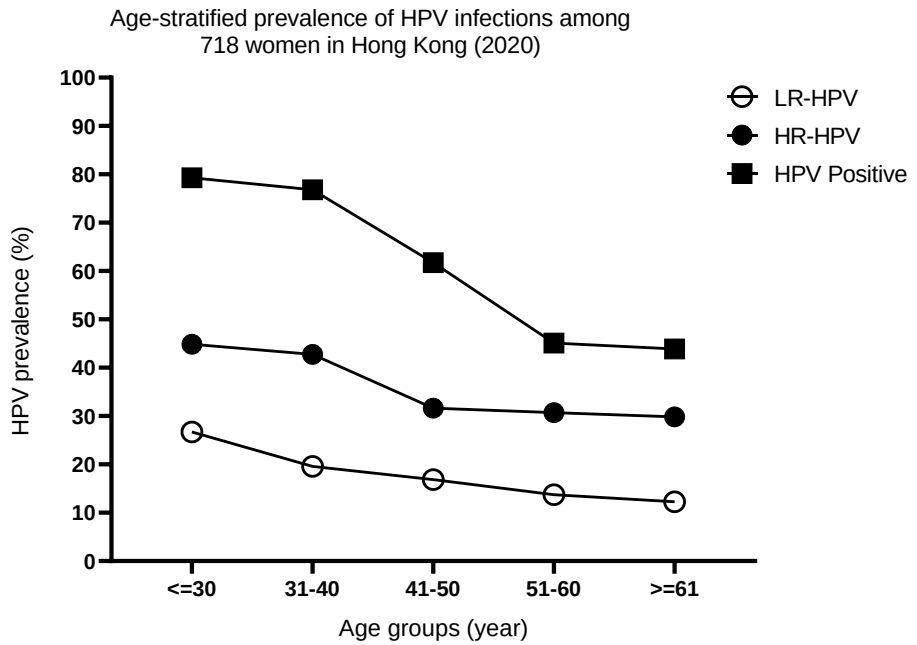

Figure 6. Age-stratified prevalence of HPV infections among 718 women in Hong Kong (2020). HPV positive indicates any HPV genotype infection, while HR-HPV and LR-HPV indicate the infections of HR-HPV and LR-HPV, respectively. In 2020, all the HPV positivity infections among the ages were in a skewed proper distribution. Using the Pearson chi-square test, the statistical significance value is $p = 0.0312$ (p-value < 0.05 is considered statistically significant).

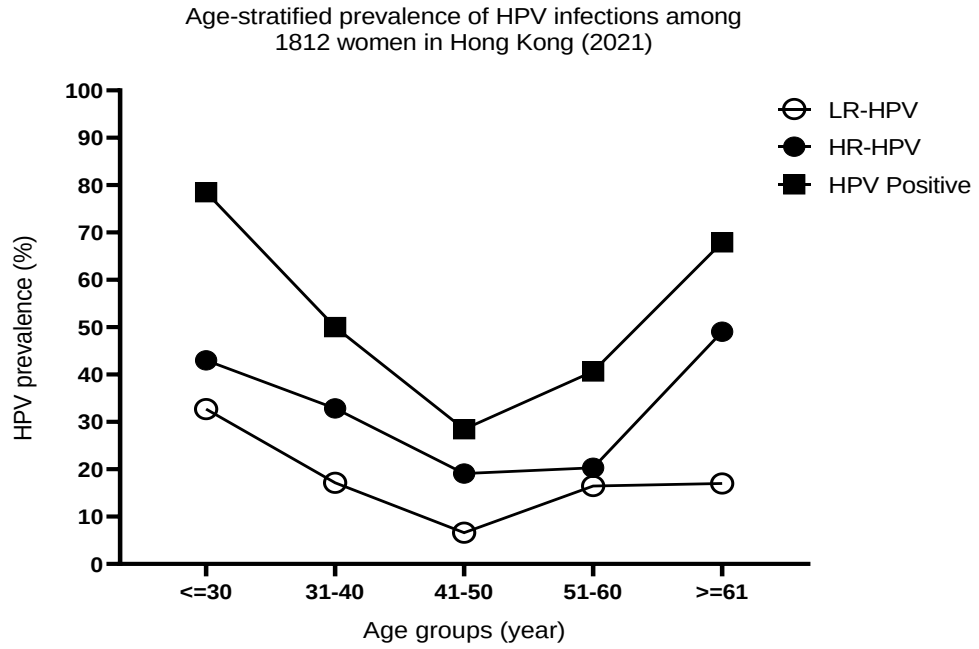

Figure 7. Age-stratified prevalence of HPV infections among 1812 women in Hong Kong (2021). HPV positive indicates any HPV genotype infection, while HR-HPV and LR-HPV indicate the infections of HR-HPV and LR-HPV, respectively. In 2021, all the HPV positivity infections among age were non-symmetric with a bimodal distribution. Using the Pearson chi-square test, the statistical significance value is $p = 0.0534$ (p-value < 0.05 is considered statistically significant).

In our study of data collected in 2020 (Figure 6), the most susceptible age group was below 30 years old, and the second infection peak was observed in the 31-40 years old, while the above 61 age group was the most HPV-resistant group. In contrast, for the analysis of data collected in 2021 (Figure 7), the most susceptible age group was still below 30 years old, and the second infection peak was changed to above 61 years old, while the 41-50 years age group was the most HPV resistant group.

Discussion

HPV vaccines

In Hong Kong, the 9-valent HPV vaccine consists of HR-HPV 16, 18, 31, 33, 45, 52, and 58, accounting for about 90% of cases of cervical cancer. The citizens should be presumptively protected from all the above seven HR-HPV genotypes under the vaccination scheme by Hong Kong Government.

The HPV vaccine is a prophylactic vaccine to prevent cervical-related cancer or diseases20. With the implementation and positive impact of the HPV vaccination scheme, a new change in the prevalence of genotypes minimizes the past and frequently prevalent subtypes of HR-HPV in Hong Kong. (e.g. HR-16, 18, 31, 33 shown in Figure 4)

Previous studies & HPV Overall Prevalence (HR/LR)

Regarding the previous studies on the prevalence of HR-HPV, this research data showed a high prevalence of HR-58 from 2018 to 2022 between Hong Kong3 and China21. However, previous research3 in 2018 analyzed 109 samples collected from a Hong Kong private laboratory, and the overall HPV-positive rate was 39.4% (43/109, n = 109). It found that the top three prevalent subtypes were HR-58 (13.4%), HR-52 (9%), and HR-59 (7.5%). Moreover, a study published in 2022 showed that the typical pattern of HPV infections caused by HR-HPV (71.8%) is associated with a higher risk of causing cervical abnormalities in China21. Additionally, the most frequent subtypes of the HR-HPV genotype were HPV-16 (4.3%), followed by HPV-52 (3.5%) and HPV-58 (2.0%)21. In contrast, in Hong Kong, HPV-16 is not in the top 1 (4.0%) between 2020 and 2021.

Generally speaking, HR-16 is the primary HPV subtype with a relatively high risk of cervical cancer development22. 70% of cervical cancers and pre-invasive cervical diseases or abnormalities are occurrences of HPV subtypes, such as HR-16 & HR-18, in the world22. HR-16 is significantly and relatively decreasing in Hong Kong compared to other countries21. Therefore, the risky HR-HPV subtypes (e.g. HR-16) have diminished recently.

However, in this research, from 2020 to 2021, the prevalence rate was 54.6% higher than 39.4% in 20183. Moreover, HR-45, HR-33, and HR-18 were the lower-ranking subtypes, resulting in a significantly decreasing trend (prevalence rate $\leq$ 2.3%). Besides, the prevalence of the abovementioned HR-HPV changed in response to the successful vaccination scheme (9-valent HPV vaccine). A total of 24,000 females received the first dose of the HPV vaccine until 31-Dec-2021, as posted in LCQ10: Human papillomavirus vaccination by HKSARS Press Releases23. The reason is highly possible due to citizens' high participation and willingness to implement the vaccination scheme officially from 2006 until the present.

Consistent with the expected protection from the HPV vaccine of the 9-valent vaccine, for the other subtypes included in the 9-valent vaccine protection, such as HR-18 and HR-31, these HR-HPV genotypes are not included in the top 10 (shown in Figure 4). The official vaccination scheme24 successfully turned down the rate of HR-18 and HR-31 to minimize the risk of further cervical abnormality development25.

Moreover, unfortunately, the most prevalent subtype in this research is HR-52 in both HPV-infected persons suffering from cervical abnormalities. Furthermore, HR-58 is in the top 3 shown in Figure 4. Besides, HR-52 is one of the most common HPV subtypes among the general population in China than in developed countries26, commonly caused in patients with cervical cancer (e.g. CIN, grade 1) or its precursors in China than in other countries27.

Therefore, in this research, the data for the prevalence of the HR-HPV subtype (HR-52) matched the global Asia studies published by China26. As a result, the effectiveness of the vaccination scheme for a positive impact of elimination for HR-HPV subtypes should be re-estimated, especially for HR-52.

Cytological Results versus HPV Infection

In this study from 2020 to 2021, all specimens, as shown in a heat map, present a significant correlation between HPV infection and cytological status in Figure 5.2.C. Previous analysis performed28 showed that the HPV positive rate was 92.1% in all ASC-US population. In this research, the HPV-positive rate correlated to ASC-US is 62%. In comparison, over the past ten years28, a decline of 30.1% by the effective vaccination has successfully provided protection for females and minimised or eradicated the HPV subtypes (HR/ LR), lowering the HPV-positive rate in Hong Kong. Therefore, a positive impact of the HPV vaccine is the outcome of achieving the presumptive expectation.

On the other hand, in Figure 5, most patients suffer from detected HPV genotypes (HR/LR), but no cytological findings result (e.g., Negative according to the Bethesda system). Poor screener performance may affect the accuracy of cytological screening results. The cytologist's observation, pre-analytical preparation of the sample site, or the number of cervical cells added variables to the diagnosis. Therefore, incorporating cytological screening and HPV-DNA test minimizes and avoids the error rate of false-negative up to 27-42%29.

Reason for HR-HPV causing cervical abnormality

Most globally published studies30,31 showed cervical abnormality correlated well with HR-HPV infection than with LR-HPV infection.

Because of genetic differences between HR-HPV (e.g., viral oncoproteins E6 and E7 for early carcinogenesis) and LR-HPV, HR-16 and HR-1832 are frequently discovered to have a significantly greater association with invasive cervical cancer than the other HPV subtypes33.

Regarding the HPV genome, E1 to E8 encode early structural genes, and L1 and L2 encode late structural genes with the help of a promoter to express (e.g., P97 and P670 in HR-16)34.

The late-coding regions yield structural proteins, while the completion of early-coding regions, including E6 and E7, is responsible for early transcription, genome amplification, and malignant transformation34,35. HR-HPVs containing E6 and E7 proteins have early and rapid transforming cellular activity, leading to independent and long-lasting activity and vice versa in LR-HPVs35.

Therefore, HPV detection plays a critical role in the diagnosis and clinical management of pre-cancerous lesions. Most likely, with HPV early detection (e.g., PCR of HPV viral DNA), the awareness of HR-HPV is higher than that of LR-HPV to prevent females from any pre-invasive cervical abnormalities. This screening is beneficial and important36.

Age-stratified prevalence of HPV infections

The most HPV-susceptible age group was ≤30 years (2020-2021), and the second infection peak was separately observed in 31-40 years (2020) and ≥61 years (2021), while the age group of ≥61 years (2020) and 41-50 years (2021) were the most HPV resistant group in this research. Some factors, like smoking, hormonal influence, and sexual habits, affect the infection rate in the population. Moreover, with the decline in HPV prevalence rate and increasing age, the specific cellular immunity

in older women is potent37.

Enhancing the cervical cancer awareness of HR-HPV infected younger women at age ≤30 years38, CDC39 suggests that cytological screening every three years or HPV test every five years, or both every five years lowers the cervical abnormality risk starting at age 21 to prevent early and pre-invasive cancerous state32 since HR-HPV related cervical abnormalities or cancers occur at a younger age (≤30 years) is higher than other cervical cancers32.

Overall Prevalence: HR-HPV and LR-HPV
Firstly, the HR-HPV genotypes, including HPV-68, HPV-58, and HPV-52, found in 2020 and 2021 had an increasing trend of 29%, 27% and 26.6%, respectively (shown in Table 2). Current data is highly matched and correlated with study40 to find the most prevalent types, such as HPV-52 (11.9%) and HPV-58 (9.3%) in Hong Kong. The trend can be seen.

Secondly, in Figure 4.1., the LR-HPV showed that the frequently detected LR-HPV genotypes, including HPV-6, HPV-26, and HPV-82, found in 2020 and 2021, had an increasing trend with 71%, 77.8% and 75%, separately (shown in Table 3). Cervical carcinomas are usually not closely correlated with LR-HPV subtype infection41. A previous study42 published that cervical cancer risk is higher in women suffering from genital warts (GW) than those without GW. In general, low-risk HPV types (6 and 11) cause GW commonly with sometimes co-infection and co-existence with high-risk HPV types is more likely42.

Therefore, the increasing trend of LR-HPV infection between 2020 and 2021 might not represent a positive impact of diminishing the purpose of LR-HPV by HPV (9-valent) vaccine scheme43. The 9-valent vaccine becomes more effective on HR-HPV, also called "oncogenic HPV" 44, and is prevalent in providing maximum health benefits to women's preventative strategy in Hong Kong.

Limitations

This present study has some limitations, such as a retrospective study and a small sampling size of diagnosed patients in this study. In the future, by using a more significant scale population, this might genuinely identify the distribution of individual HPV genotype(s) to assess the cervical pre-invasive abnormality of specific HR-HPV or LR-HPV types and to reflect the accurate picture of the community by using risk-stratification model for particular age group as very well.

Lastly, the collected data shows a high correlation between cervical cytological findings and pathological diagnosis, indicating that this study is reliable.

Conclusion

This study revealed the increasing HPV infection rate and genotype distribution among women aged 30 years or below, especially for HR-52 and HR-81 as the dominant subtype with a higher risk of causing cervical abnormality in recent years in Hong Kong, which could serve as vital and statistical foundation for considering HPV vaccination and preventative strategies to minimize the risk for cervical pre-invasive disease.

Last but not least, this research showed the prevalence of age-stratified HPV genotypes with a close correlation with globally published scientific data, indicating our results are accurate and reliable. A significant decline of 30.1% HPV positive rate in the ASC-US population, a success with the diminishing prevalence rate of HR-18 and HR-31, and a positive impact of the HPV vaccine is the outcome to meet the presumptive expectation.

Finally, this study provides positive impact data to reveal the effectiveness of HPV vaccination in improving the HPV-related national immunization program and

management guidelines for new patterns of individual HPV subtypes, such as HR-52, which is the predominant one.

References

1. Steben M, Duarte-Franco E. Human papillomavirus infection: epidemiology and pathophysiology. Gynecol Oncol. 2007;107:2–5. Available from: https://pubmed.ncbi.nlm.nih.gov/17938014/
2. Thomsen LT, Frederiksen K, Munk C, Junge J, Castle PE, Iftner T, Kjaer SK. High-risk and low-risk human papillomavirus and the absolute risk of cervical intraepithelial neoplasia or cancer. Obstet Gynecol. 2014;123(1):57-64. doi: 10.1097/AOG.0000000000000056. PMID: 24463664.
3. Lu YC, Chung KO, Ng WS, Tam CC. Distribution of human papillomavirus types in local population with cytological abnormalities. J Hong Kong Inst Med Lab Sci. 2018;15(1&2). Available from: https://bit.ly/3tmLB5M
4. Nayar R, Wilbur DC. The Pap Test and Bethesda 2014. Acta Cytol. 2015;59(2):121–32. Available from: https://acsjournals.onlinelibrary.wiley.com/doi/10.1002/cncy.21521
5. Bruni L, Albero G, Serrano B, Mena M, Collado JJ, Gómez D, Muñoz J, Bosch FX, de Sanjosé S. Human Papillomavirus and Related Diseases in the World. Summary Report. ICO/IARC Information Centre on HPV and Cancer; 2022. Available from: https://hpvcentre.net/statistics/reports/XWX.pdf
6. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis. 2010;202(12):1789–99. doi: 10.1086/657321.
7. Vinodhini K, Shanmughapriya S, Das BC, Natarajaseenivasan K. Prevalence and risk factors of HPV infection among women from various provinces of the world. Arch Gynecol Obstet. 2012;285:771–7. doi: 10.1007/s00404-011-2155-8.
8. Asiaf A, Ahmad ST, Mohammad SO, Zargar MA. Review of the current knowledge on the epidemiology, pathogenesis, and prevention of human papillomavirus infection. Eur J Cancer Prev. 2014;23:206–24. doi: 10.1097/CEJ.0b013e328364f273. Available from: https://pubmed.ncbi.nlm.nih.gov/24129107/
9. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of human papillomavirus–positive head and neck squamous cell carcinoma. J Clin Oncol. 2015;33(29):3235-42. Available from: https://pubmed.ncbi.nlm.nih.gov/26351338/
10. Kombe Kombe AJ, Li B, Zahid A, Mengist HM, Bounda GA, Zhou Y, Jin T. Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation. Front Public Health. 2021;8:552028. doi: 10.3389/fpubh.2020.552028.
11. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. The Lancet Oncol. 2010;11(11):1048–56. doi:10.1016/S1470-2045(10)70230-8.
12. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings.

J Infect Dis. 2010;202(12):1789–99. doi:10.1086/657321.

13. Fehrmann F, Laimins LA. Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. Oncogene. 2003;22:5201–7. Available from: https://pubmed.ncbi.nlm.nih.gov/12910257/.

14. Graham SV, Faizo AAA. Control of human papillomavirus gene expression by alternative splicing. Virus Res. 2017;231:83–95. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5335905/.

15. Groves IJ, Drane ELA, Michalski M, Monahan JM, Scarpini CG, Smith SP, et al. Short- and long-range cis interactions between integrated HPV genomes and cellular chromatin dysregulate host gene expression in early cervical carcinogenesis. PLoS Pathog. 2021;17(8):e1009875. doi:10.1371/journal.ppat.1009875.

16. Wooldridge TR, Laimins LA. Regulation of human papillomavirus type 31 gene expression during the differentiation-dependent life cycle through histone modifications and transcription factor binding. Virology. 2008;374(2):371–80. Available from: https://pubmed.ncbi.nlm.nih.gov/18237759/.

17. Wang X, Song Y, Wei X, Wang G, Sun R, Wang M, et al. Prevalence and distribution of human papillomavirus genotypes among women attending gynaecology clinics in northern Henan Province of China. Virology J. 2022;19:6. doi:10.1186/s12985-021-01732-8.

18. Cancer attributable to infections International Agency for Research on Cancer (IARC). GLOBOCAN. 2018. Available from: https://gco.iarc.fr/causes/infections/tools-pie?mode=2&sex=2&population=hdi&continent=0&country=0&cancer=0&key=attr_cases&lock_scale=0&pie_mode=1&nb_results=5.

19. Chau MY, Chung KO, Ng WS, Tam CC. Prevalence of human papillomavirus infection and its correlation with age and cervical cytology results in Hong Kong. J Hong Kong Inst Med Lab Sci. 2018;15(1 & 2):50–9. Available from: https://bit.ly/3tmLB5M.

20. Centre for Health Protection, Department of Health, HKSARS, CHP. Vaccination schemes: About human papillomavirus (HPV) vaccination. 2022. Available from: https://www.chp.gov.hk/en/features/102146.html.

21. Wang X, Song Y, Wei X, Wang G, Sun R, Wang M, et al. Prevalence and distribution of human papillomavirus genotypes among women attending gynaecology clinics in northern Henan Province of China. Virology J. 2022;19:6. doi:10.1186/s12985-021-01732-8.

22. WHO. Human papillomavirus (HPV) and cervical cancer 2015. Available from: http://www.who.int/mediacentre/factsheets/fs380/en/.

23. HKSARS Press Releases. LCQ10: Human papillomavirus vaccination. 2021. Available from: https://www.info.gov.hk/gia/general/202101/20/P2021012000507.htm.

24. Centre for Health Protection, Department of Health, HKSARS, CHP. Vaccination schemes: About human papillomavirus (HPV) vaccination. 2022. Available from: https://www.chp.gov.hk/en/features/102146.html.

25. Burd E. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003;16(1):1–17. doi:10.1128/CMR.16.1.1-17.2003.

26. Jiang L, Tian X, Peng D, Zhang L, Xie F, Bi C, et al. HPV prevalence and genotype distribution among women in Shandong Province, China: Analysis of 94,489 HPV

genotyping results from Shandong's largest independent pathology laboratory. PLoS ONE. 2019;14(1):e0210311. doi:10.1371/journal.pone.0210311.

27. Zhang J, Cheng K, Wang Z. Prevalence and distribution of human papillomavirus genotypes in cervical intraepithelial neoplasia in China: a meta-analysis. Arch Gynecol Obstet. 2020;302(6):1329–37. https://doi.org/10.1007/s00404-020-05787-w

28. Ng WS, Lo TH, Tam CC. Correlation of HPV types in archived ASCUS samples. J Hong Kong Inst Med Lab Sci. 2011;12(1&2). http://www.hkimls.org/j2010cont.htm

29. Evans KK, Tambouret RH, Evered A, Wilbur D, Wolfe JM. Prevalence of abnormalities influences cytologists' error rates in screening for cervical cancer. Arch Pathol Lab Med. 2014;135(12):1557–60. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3966132/

30. Sangrajrang S, Laowahutanont P, Wongsena M, Muwonge R, Karalak A, Imsamran W, et al. Comparative accuracy of Pap smear and HPV screening in Ubon Ratchathani in Thailand. Papillomavirus Res. 2017;3:30–5. https://doi.org/10.1016/j.pvr.2016.12.004

31. Yu Y, Jiang M, Dang L, Feng R, Bangura MS, Chen W, Qiao Y. Changes in high-risk HPV infection prevalence and associated factors in selected rural areas of China: a multicenter population-based study. Front Med. 2022. https://doi.org/10.3389/fmed.2022.911367

32. Pinkowish MD. Human papillomavirus genotype distributions inform screening and vaccination policy. CA Cancer J Clin. 2009;59:280–1. https://acsjournals.onlinelibrary.wiley.com/doi/full/10.3322/caac.20035

33. Yim EK, Park JS. The role of HPV E6 and E7 oncoproteins in HPV-associated cervical carcinogenesis. Cancer Res Treat. 2005;37(6):319–24. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2785934/

34. Zheng ZM, Baker CC. Papillomavirus genome structure, expression, and post-transcriptional regulation. Front Biosci. 2006;11(3):2286–302. doi:10.2741/1971.

35. Psyrri A, Boutati E, Karageorgopoulou S. Human papillomavirus in head and neck cancers: biology, prognosis, hope of treatment, and vaccines. Anticancer Drugs. 2011;22(7):586–90. Available from: https://pubmed.ncbi.nlm.nih.gov/21403517/.

36. Nahar F, Hossain MA, Paul SK, Ahmed MU, Khatun S, Bhuiyan GR, Nasreen SA, Haque N, Ahmed S, Kobayashi N, Akter SN, Begum H. Molecular Diagnosis of Human Papilloma Virus by PCR. Mymensingh Med J. 2019;28(1):175–81. Available from: https://pubmed.ncbi.nlm.nih.gov/30755567/.

37. Burk R, Kelly P, Feldman J, Bromberg J, Vermund SH, DeHovitz JA, Landesman SH. Declining prevalence of cervicovaginal human papillomavirus infection with age is independent of other risk factors. Sex Transm Dis. 1996;23(4):333–41. Available from: https://pubmed.ncbi.nlm.nih.gov/8836027/.

38. Cheung TH, Cheng SSY, Hsu DC, Wong WL, Pavelyev A, Walia A, Saxena K, Prabhu VS. The impact and cost-effectiveness of 9-valent human papillomavirus vaccine in adolescent females in Hong Kong. Cost Eff Resour Alloc. 2021;19:75. doi:10.1186/s12962-021-00328-x.

39. Centers for Disease Control and Prevention (CDC). Cervical Cancer Awareness: Screening Tests and Options.

2021. Available from: https://www.cdc.gov/cancer/dcpc/resources/features/cervicalcancer/index.htm .

40. Lau YM, Cheung TH, Yeo W, Mo F, Yu MY, Lee KM, Ho CS, Yeung CM, Law TY, Chan KS. Prognostic implication of human papillomavirus types and species in cervical cancer patients undergoing primary treatment. PLoS ONE. 2015;10(4):e0122557. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0122557.

41. Thomsen LT, Frederiksen K, Munk C, Junge J, Castle PE, Iftner T, Kjaer SK. High-risk and low-risk human papillomavirus and the absolute risk of cervical intraepithelial neoplasia or cancer. Obstet Gynecol. 2014;123(1):57–64. doi:10.1097/AOG.0000000000000056.

42. Blomberg M, Friis S, Munk C, Bautz A, Kjaer SK. Genital warts and risk of cancer: A Danish study of nearly 50,000 patients with genital warts. J Infect Dis. 2012;205(10):1544–53. doi:10.1093/infdis/jis228.

43. Centre for Health Protection, Department of Health, HKSARS, CHP. Vaccination schemes: About human papillomavirus (HPV) vaccination. 2022. Available from: https://www.chp.gov.hk/en/features/102146.html.

44. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer. 2017;141(4):664–70. doi:10.1002/ijc.3071.

The synergistic antimicrobial activity of locally sourced Hong Kong honey and lemon juice against foodborne pathogens: a pilot study

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Abstract

This pilot study aimed to assess the antibacterial properties and combined effects of two locally sourced honey varieties, fresh lemon juice and a honey-lemon juice blend, against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). Plate count analysis indicated that *S. aureus* exhibited increased susceptibility to lemon juice compared to *E. coli*. The research highlighted lemon juice's efficacy as an antibacterial agent against gram-positive bacteria like *S. aureus* over gram-negative strains such as *E. coli* in vitro. Noteworthy was the superior antibacterial performance of honey against *E. coli* in contrast to *S. aureus*. Furthermore, our study illustrated the synergistic antibacterial action of lemon juice and honey on *E. coli*. However, further investigation is needed to unveil the combined antibacterial impact of locally available honey and lemon juice on diverse pathogens.

Keywords: *Antimicrobial activity; foodborne pathogens; honey; lemon juice*

Introduction

The prevalence of drug-resistant bacteria is increasing worldwide due to the overuse of antibiotics. With the continuing development of drug-resistant bacteria, the discoveries of novel antibacterial drugs and components become crucial for treating infections caused by drug-resistant bacteria. In recent years, the broad screening of chemicals from natural products for antibacterial activity has been an alternative strategy for developing novel antibacterial drugs.

There is an increase in studies revealing the antibacterial activity of honey on various pathogens. Among those, manuka honey is one of the most popular kinds of honey, and many studies have demonstrated its antibacterial activity based on its unique antibacterial ingredients, such as methylglyoxal (MGO)[1]. On the other hand, many studies have shown that lemon juice can inhibit bacterial growth, including Staphylococcus aureus (S. aureus) and *Escherichia coli* (*E. coli*). Lemon and Lime juices have been reported to exhibit antibacterial activity against *S. aureus, E. coli*, and other food-born bacterial pathogens in cooked foods.[2] Lemon is a popular citrus fruit and food ingredient for flavouring and adding acidity. Lemon is also commonly mixed with honey to enhance its flavour. It is one of the most popular drinks in Hong Kong, particularly in the winter season. However, there has been no study on the antibacterial activities of using locally available honey. No study has investigated the synergistic antibacterial effect of lemon juice and honey mixture on foodborne pathogens such as S. aureus and *E. coli.* Understanding the synergistic antibacterial effects of lemon juice and honey can have practical implications for food processing and preservation industries. It may lead to the development of natural antibacterial agents for enhancing food safety and extending shelf life. This pilot study explored the antibacterial activities and synergistic antibacterial effects of two kinds of honey available in Hong Kong, fresh lemon juice and honey lemon juice mixture against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922).

Materials and Methods

Preparation of Honey

Two kinds of honey, Winter Honey (WH) and Supreme Honey (SH), originating from Hong Kong, were selected for this study. The two kinds of honey were 100% pure and stored at room temperature. Each was prepared at 75% v/v concentration with sterile distilled water at a final volume of 1 mL.

Preparation of fresh Lemon juice

Fresh lemons from China were purchased from the local supermarket. The juice was squeezed out of the lemons and pooled into the sterilized bottle. The juice was stored in the refrigerator at 4°C for subsequent use.

Preparation of mixed honey and fresh Lemon juice

Honey and lemon juice were combined in equal parts by weight and volume, with 0.5g of 100% honey mixed with 0.5 mL of 100% lemon juice, using a vortex mixer. The mixture was then stored in an Eppendorf tube for future use.

Bacteria strains and the preparation of growth curves

Two bacteria strains, Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923), were purchased from ATCC. Stock bacterial broth cultures of 0.5 McFarland were prepared using normal saline as the original bacterial concentration. The growth curves for both bacterial strains were established from the 0.5 McFarland cultures for use in subsequent experiments.

Measurement of the antibacterial effect: plate count method

10µl of 0.5 McFarland bacteria suspensions were mixed with 190µl of the following six combined sample solutions: Winter honey (WH); Supreme honey (SH); Lemon juice; Winter honey and lemon juice; Supreme honey and lemon juice; and normal saline (as neat control). The 200µl samples were grown in nutrient broth at 37°C for 0-4 hours. Samples were collected at different time points, and optical density (OD) was measured at 600nm. 100µL of the sample was extracted and subjected to serial dilution in a nutrient broth. Subsequently, 10µL from each dilution was spread onto nutrient agar plates for further analysis.[3] The agar plate was incubated at 37°C overnight and the number of colonies was counted to determine the survival fractions as the colony-forming units (CFUs).

Statistical analysis

Data were collected from at least three independent experiments and findings were presented as mean±SD. Two-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was performed, and differences were considered statistically significant with $p \leq 0.05$.

Results

Figures 1 and 2 demonstrated the correlation between bacterial colony number and the optical density (OD) measured as absorbance at 600nm.

The plate count method measured the antibacterial activities of two honeys, lemon juice, and the mixtures of honey and lemon juice.

For *E. coli*, compared to normal saline, there was a significant decrease in the number of bacteria when treated with lemon juice, Supreme honey, Winter honey, a 1:1 mixture of the lemon juice and Supreme honey or a 1:1 mixture of the lemon juice and Winter honey. Among the tested conditions, the lemon mixed with Supreme honey resulted in the greatest decrease in the log number of bacteria, with 2.64 log bacteria decreasing after 2 hours and reaching zero bacteria colony detected after 4 hours. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$)

Similar results were obtained for *S. aureus*, compared to normal saline, with a significant decrease in the number of bacteria observed when treated with lemon juice, a 1:1 mixture of the lemon juice and Supreme honey or a 1:1 mixture of the lemon juice and Winter honey. Among the tested conditions, the lemon juice, 1:1 mixture of the lemon juice and Supreme honey or 1:1 mixture of the lemon juice and Winter honey reached zero bacteria colonies detected after 4 hours. (** $P<0.01$, *** $P<0.001$)

Discussion

Based on the plate count findings, the data indicates that *S. aureus* exhibits greater susceptibility to lemon juice than *E. coli*. The resistance of *E. coli* to lemon juice is due to its unique acid-resistant characteristics, enabling it to endure and thrive in acidic environments.[4] Our finding was echoed by Fisher and Phillip's study, with results showing that lemon juice is a practical anti-bacterial component to gram-positive bacteria (e.g. *S. aureus*) than the gram-negative bacteria (e.g. *E. coli*) *in vitro.*[5]

Interestingly, honey demonstrated better antibacterial activity on *E. coli* than *S. aureus.* Among the two kinds of honey tested, Winter honey showed better antibacterial activity than Supreme honey on both *E. coli* and *S. aureus*. This difference may be because the concentration of hydrogen peroxide in Winter honey is more abundant than in Supreme honey. Hydrogen peroxide is produced by the enzyme glucose oxidase, which is naturally present in honey. Hydrogen peroxide contributes to honey's ability to inhibit the growth of certain bacteria and fungi. The low water activity and acidic pH of honey also create an unfavourable environment for microbial growth.[6] Karabagias et al. demonstrated that the antibacterial activity of honey depends on the flower source, honey

bees, and the processing method between different types of honey.[7] Escuredo's studies also explained that the physical properties of honey, such as sugar content, pH value, water activity, and presence of phenol compound and/or active amino acid (eg. kynurenic acid), can also contribute to the antibacterial effect.[8,9] Our study also demonstrated the synergistic antibacterial activity between lemon juice and honey on *E.coli.* However, further investigation is needed to reveal the synergistic antibacterial effect of locally available honey and lemon juice on different pathogens.

Conclusion

This pilot study demonstrated the antibacterial activity of lemon juice, honey available in Hong Kong, and the mixture of lemon juice and honey against *E. coli* and *S. aureus.* Further mechanistic investigation is needed to reveal the synergistic antibacterial effect of honey and lemon juice on *S. aureus* and *E. coli*.

References

1. Adams CJ, Boult CH, Deadman BJ, Farr JM, Grainger MC, Manley-Harris M, Snow MJ. Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (Leptospermum scoparium) honey. Carbohydr Res. 2008;343(4):651–9.
2. Presentato A, Scurria A, Albanese L, Lino C, Sciortino M, Pagliaro M, Zabini F, Meneguzzo F, Alduina R, Nuzzo D, Ciriminna R. Superior antibacterial activity of integral lemon pectin extracted via hydrodynamic cavitation. ChemistryOpen. 2020;9(5):628–30.
3. Tang HM, Hamblin MR, Yow CM. A comparative in vitro photoinactivation study of clinical isolates of multidrug-resistant pathogens. J Infect Chemother. 2007;13(2):87–9.
4. Greg B, Chai-Hoi, Ann I. Acid tolerance of Escherichia coli following cold shock treatment. J Food Saf. 2007;23:2.
5. Fisher K, Phillips CA. The effect of lemon, orange and bergamot essential oils and their components on the survival of Campylobacter jejuni, Escherichia coli O157, Listeria monocytogenes, Bacillus cereus and Staphylococcus aureus in vitro and in food systems. J Appl Microbiol. 2006;101(6):1232–40.
6. Alaerjani WMA, Abu-Melha S, Alshareef RMH, et al. Biochemical reactions and their biological contributions in honey. Molecules. 2022;27(15):4719.
7. Karabagias IK, Badeka A, Kontakos S, et al. Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. Food Chem. 2014;146:548–57.
8. Escuredo O, Míguez M, Fernández-González M, Seijo MC. Nutritional value and antioxidant activity of honey produced in a European Atlantic area. Food Chem. 2013;138(2):851–6.
9. Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci. 2013;16(6):731–42.

Figure. 1 Determine the relationship between the number of bacteria and absorbance on *E.coli* (ATCC 25922)

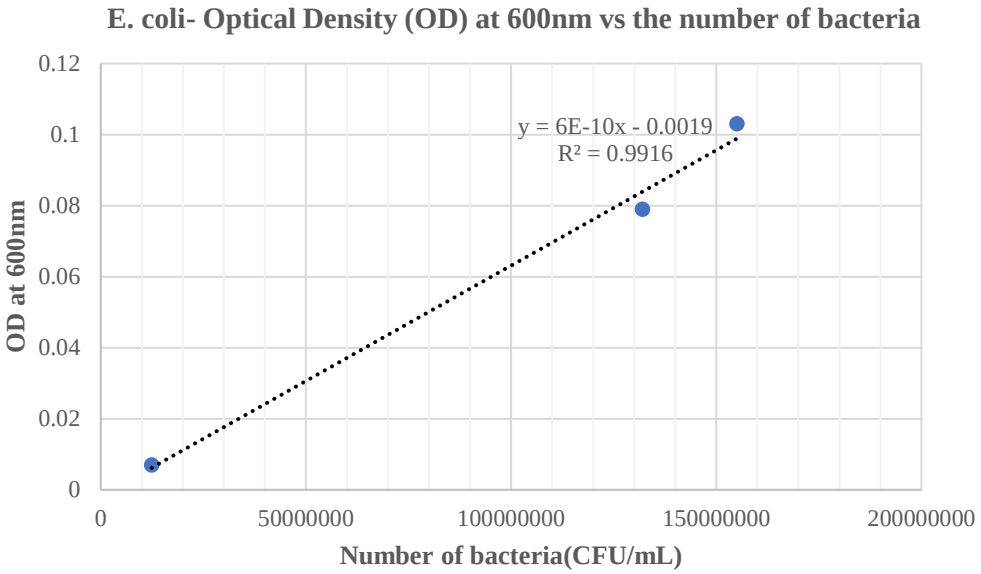

Figure 1 shows the relationship between the number of *E. coli* colony-forming units and absorbance (OD) measured at 600nm. Samples were plated to count the number of bacterial colonies.

Figure. 2 Determine the relationship between the number of bacteria and absorbance on *S. aureus* (ATCC 25923)

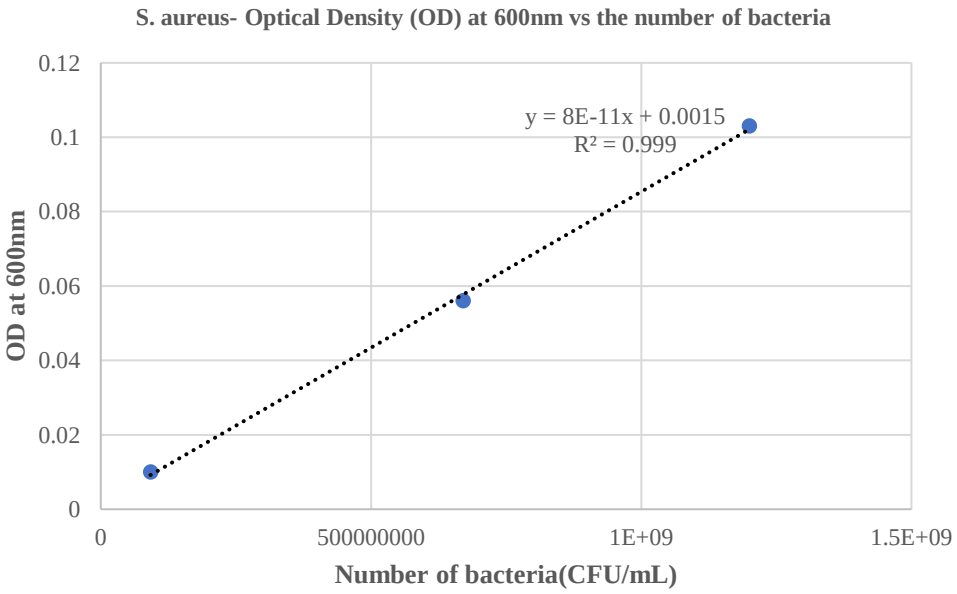

Figure 2 shows the relationship between the number of *S. aureus* colony-forming units and absorbance (OD) measured at 600nm. Samples were plated to count the number of bacterial colonies.

Figure. 3 Log number of bacteria vs time on *E. coli* (ATCC 25922)

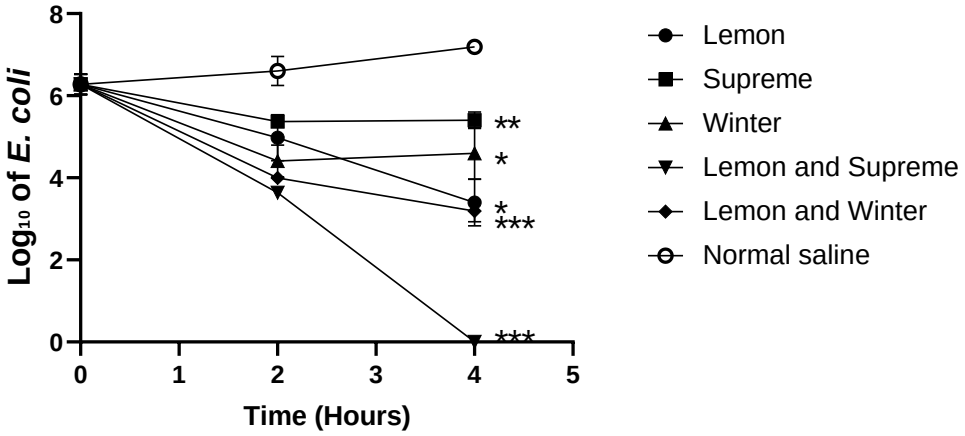

Figure 3 shows the survival curve of *E. coli* treated with lemon juice and two kinds of honey. The strain was incubated with 100% lemon juice, 75% Supreme honey, 75% Winter honey, 1:1 mixture of lemon juice and Supreme honey, 1:1 mixture of lemon juice and Winter honey. The normal saline was the negative control of the experiment. The bacterial colonies were counted at 2 and 4 hours of incubation time. Three values were collected for each time point from 3 independent experiments.

Figure. 4 Log number of bacteria vs time on *S. aureus* (ATCC 25923)

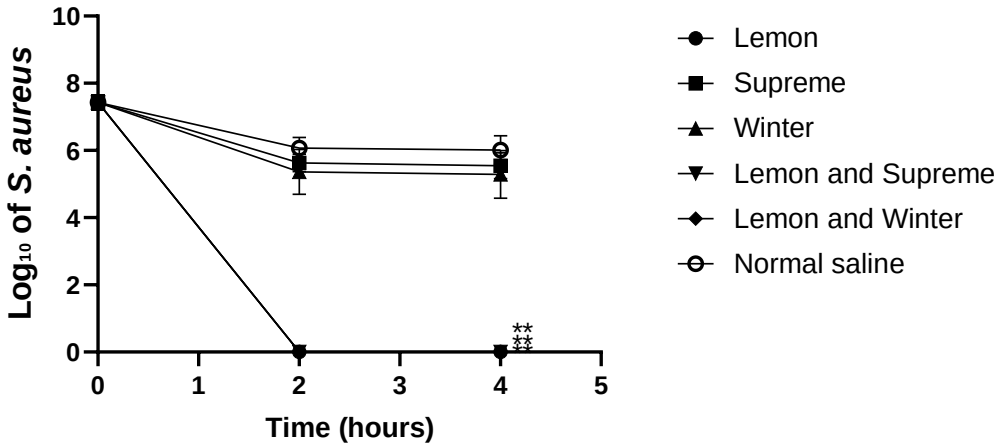

Figure 4 shows the survival curve of *S. aureus* treated with lemon juice and two selected honey. The strain was incubated with 100% lemon juice, 75% Supreme honey, 75% Winter honey, 1:1 mixture of lemon juice and Supreme honey, 1:1 mixture of lemon juice and Winter honey. The normal saline was the negative control of the experiment. The bacterial colonies were counted at 2 and 4 hours of incubation time. Three values were collected for each time point from 3 independent experiments.

HbA1c is a good indicator for predicting dyslipidemia.

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Abstract

This study explored the association between Glycosylated Hemoglobin A1c (HbA1c) and Lipid Profiles, alongside their clinical implications, using statistical analysis methods. An examination was made on data from 1000 subjects, encompassing HbA1c levels, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), as well as demographic and clinical variables such as age, gender, and body mass index (BMI). The outcomes of this investigation demonstrate a significant relationship between HbA1c and various lipid parameters circulating in the body. Noteworthy is the positive and substantial correlation between HbA1c and total cholesterol and triglycerides, suggesting a link between glycemic regulation and dyslipidemia. Age, gender, and BMI were pinpointed as factors influencing lipid profiles and HbA1c levels, with older individuals, females, people with obesity, and those with diabetes being at an increased risk of cardiovascular disease development due to the dyslipidemia condition. In summary, HbA1c emerges as a reliable gauge for glycemic management and a valuable tool for predicting dyslipidemia in diabetic patients. Consequently, monitoring glycemic indicators can enhance the early identification of dyslipidemia. Thus, it is strongly advised to regularly monitor blood glucose levels, HbA1c, and lipid profiles for comprehensive health evaluations.

Keywords: *Kidney Stones; Urine Analysis; Predictive Model; Data-Driven Approach*

Introduction

Diabetes mellitus (DM) stands as a prevalent metabolic disorder worldwide, significantly contributing to both morbidity and mortality rates. In 2022, approximately 10,399 inpatient discharges and deaths were linked to DM, underscoring its profound impact[1]. The ramifications of poor glycemic control in diabetes extend to increased risks of dyslipidemia and vascular diseases, culminating in long-term organ damage and dysfunction. While Type 1 diabetes affects 5% of diagnosed cases and demands meticulous glycemic oversight to avert abnormal lipid profiles, Type 2 diabetes (T2DM) accounts for 95% of cases. It is intricately tied to insulin resistance and metabolic dysregulation, often associated with cardiovascular complications[2].

Efficient management of diabetes through judicious lifestyle choices, exercise, and medication can mitigate severe complications like end-stage renal disease, blindness, and neuropathy[3]. Hemoglobin A1c (HbA1c) emerges as a pivotal marker for glycemic control and diabetes diagnosis, reflecting glucose concentrations over the preceding three months. HbA1c testing presents a convenient alternative to the oral glucose tolerance test, obviating the necessity for fasting periods to minimise variability[4]. The metric holds predictive value for assessing long-term diabetic complications based on multi-year data and finds common usage in screening for gestational diabetes among pregnant women.

Recent research indicates that HbA1c not only acts as a glycemic control marker but also serves as a valuable predictor of lipid profiles, aiding in the identification of individuals at high risk for Cardiovascular diseases[4].

Recent studies highlight HbA1c as a glycemic control marker and a predictor of lipid profiles, aiding in identifying individuals at high cardiovascular risk. Various risk factors like gender, age, and BMI influence the relationship between HbA1c and lipid profiles, emphasising further research to understand the precise risk of dyslipidemia development based on HbA1c levels. Exploring these aspects comprehensively can enhance clinical strategies for managing glycemic and cholesterol levels in diabetic patients.

Direct correlations have been observed between HbA1c and total cholesterol, triglycerides, and LDL-C, with an inverse relationship with HDL-C, particularly in individuals with inadequate glycemic control[5]. However, conflicting findings exist, with some studies indicating no correlation between HbA1c and HDL-C[6] or lipid profiles in general[7]. Elevated HbA1c levels, significantly exceeding 8%, are associated with heightened risks of mortality in diabetes and CVDs, highlighting the interconnected nature of diabetes and dyslipidemia in accelerating atherogenesis.[8,9]

Given the discrepancies in the relationship between HbA1c and lipid parameters, further research is imperative to explore potential variations and ascertain the risk of developing dyslipidemia based on HbA1c levels. A comprehensive investigation of these factors can elucidate the association between glycemic control, lipid profiles, and cardiovascular risk, assisting clinicians in refining their focus on managing glycemic and cholesterol levels in diabetic patient.

Material and Method

Data Source and Description

The dataset was procured from an online repository, specifically Kaggle, and predominantly comprises data derived from the Iraqi population. Data gathering involved retrieving patients' records from the laboratories of Medical City Hospital and the Specialized Center for Endocrinology and Diabetes at Al-Kindy Teaching Hospital. A cohort of 1000 participants aged between 20 and 80 years was included, comprising 435 women and 565 men. Demographic information such as age and gender, along with clinical data like BMI, was collected. Additionally, biological parameters, including HbA1c, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG), were incorporated for risk factor and subgroup analyses. HbA1c values were a percentage of total haemoglobin, while lipid parameter values were expressed in mmol/L.

Definition and Reference Range Setting

Hemoglobin A1c (HbA1c) serves as a dependable diagnostic marker for diabetes, endorsed by global bodies like the World Health Organization (WHO) and the American Diabetes Association (ADA). The standardisation of HbA1c dictates that results should be reported in two units: a conventional percentage unit using the National Glycohemoglobin Standardization Program (NGSP)-certified method aligned with the Diabetes Control and Complications Trial Assay and an SI unit (mmol/mol) as per the International Federation of Clinical Chemistry (IFCC) guidelines[3]. Non-diabetic individuals typically exhibit HbA1c levels falling within the range of 4.0% to 6.4%. A threshold of 6.5% (48 mmol/mol) is used to diagnose diabetes mellitus in adults as an alternative to the fasting plasma glucose criterion of ≥7.0 mmol/L[10].

The National Cholesterol Education Program (NCEP) Adult Treatment Panel III guidelines were consulted for serum lipid reference levels. Dyslipidemia, a lipid metabolism disorder, may stem from elevated total cholesterol, high low-density lipoprotein cholesterol, low high-density lipoprotein cholesterol, or increased triglyceride levels. As outlined in the guidelines, dyslipidemia is indicated by total cholesterol levels exceeding >5.18 mmol/L (>200 mg/dL), fasting triglycerides surpassing >1.7 mmol/L (>150 mg/dL), low HDL-C levels below <1.04 mmol/L (<40 mg/dL), and high LDL-C levels above >3.37 mmol/L (>130 mg/dL), presenting increased cardiovascular disease risk factors[11].

According to the World Health Organization's Regional Office for the Western Pacific in 2000, body mass index (BMI) classifications are as follows: individuals with a BMI less than 18.5 kg/m² are categorised as underweight, those falling between 18.5 and 22.9 are within the normal weight range, individuals with BMIs between 23 and 24.9 are considered overweight, and those with BMIs equal to or exceeding 25 are classified as obese[12].

Statistical Method

Correlation analysis evaluated the strength and direction of the relationship between HbA1c and lipid profiles. Pearson's correlation coefficient was utilised to assess the diverse correlations among these variables. Pearson’s correlation test examined correlations between continuous variables such as HbA1c, cholesterol, triglycerides, HDL-C, and LDL-C. An Independent sample t-test was performed to compare means across different parameters.

Subgroup analysis was conducted to investigate potential variations in the association between HbA1c and lipid profiles

based on specific characteristics or subpopulations. Four distinct subgroups were delineated, including Diabetes and non-diabetes, Female and Male, Age categories of 20-40, 41-60, and 61-80, and BMI classifications (Underweight, Normal-weight, Overweight, and Obese). Unpaired student's t-tests and one-way ANOVA tests were utilised for subgroup comparisons. Pearson's correlation coefficient was applied within each group to ascertain the direction and strength of correlations.

All the datasets underwent analysis utilising Prism GraphPad 9, where descriptive statistics were employed to provide an overview of the dataset's characteristics. A p-value below 0.05 was deemed statistically significant, while a p-value below 0.01 was considered statistically highly significant.

Ethical considerations

The dataset sourced from Kaggle[13] was duly acknowledged.

Results

Patient's Demographic data

The study consisted of 1000 patients, with detailed demographic and clinical information in Table 1. Among the participants, 435 were female, and 565 were male, all of whom were adults, with a mean age of 54 and a standard deviation of 8.8. Most individuals were 41-60 years old, accounting for 75.1% of the total cohort.

None of the subjects were categorised as underweight, while 9.3% were classified as normal weight, 9.7% as overweight, and the majority, comprising 81% of the population, were identified as obese. The prevalence of non-diabetic individuals was 23.9%, whereas 76.1% (n=761) were diagnosed with diabetes mellitus.

The study unveiled that hypercholesterolemia was a prevalent lipid abnormality, impacting 62% of the patient cohort. Additionally, hypertriglyceridemia, reduced HDL-C levels, and elevated LDL-C levels were identified in 62.5%, 52.8%, and 76.5% of the participants, respectively.

Correlation between HbA1c and Lipid Profiles

In our study, a strong and statistically significant association was observed between HbA1c, TC ($r = 0.18$, $p \leq 0.001$), and TG ($r = 0.22$, $p \leq 0.001$). Furthermore, HbA1c displayed notable correlations with age ($r = 0.38$, $p \leq 0.001$) and BMI ($r = 0.41$, $p \leq 0.001$). However, no significant correlation was detected between LDL-C ($r = 0.01$, $p \leq 0.001$) and HDL-C ($r = 0.03$, $p \leq 0.001$) with HbA1c. Age exhibited direct correlations with TC ($r = 0.04$, $p \leq 0.001$), LDL-C ($r = 0.02$, $p \leq 0.001$), and BMI ($r = 0.38$, $p \leq 0.001$). In contrast, BMI was positively associated with TG ($r=0.11$, $p \leq 0.001$) and negatively correlated with LDL-C ($r = -0.07$, $p=0.033$).

Significant relationships were identified among the lipid profiles. TC demonstrated significant correlations with three other parameters: TG ($r = 0.32$, $p \leq 0.001$), HDL-C ($r = 0.1$, $p=0.001$), and LDL-C ($r = 0.42$, $p \leq 0.001$). Conversely, HDL-C exhibited inverse correlations with TG ($r = -0.08$, $p=0.009$) and LDL-C ($r = -0.14$, $p \leq 0.001$). See Table 2 for details.

Subgroup Analysis

Diabetes and non-Diabetes

Patients were divided into two groups based on their glycemic index: HbA1c levels below 6.5% (non-diabetes) and those equal to or greater than 6.5% (diabetes)[10]. Among individuals with diabetes, the majority were obese (96%) and showed a tendency for hypercholesterolemia and hypertriglyceridemia, as illustrated in Table 3.

Patients exhibiting HbA1c levels exceeding 6.5% demonstrated significantly elevated levels of TC (4.965±1.3 vs 4.536±1.3, $p<0.0001$), TG (2.504±1.5 vs 1.859±0.99,

p<0.0001), age (55.71±7.3 vs 46.58±9.7, p<0.0001), and BMI (30.98±4.1 vs 25.11±4.8, p<0.0001). Conversely, no significant variances were observed in HDL-C (1.209±0.71 vs 1.191±0.48, p=0.7208) and LDL-C (2.619±1.1 vs 2.58±1, p=0.6352) levels between the diabetes and non-diabetes groups.

Age

The significant variations observed among different age groups in some key metabolic parameters like TG (p<0.0001), LDL-C (p=0.0164), HbA1c (p<0.0001), and BMI (p<0.0001) (shown in Table 4) likely stem from a complex interplay of factors. Increasing age brings about physiological changes that can directly impact lipid levels, glucose regulation, and body mass index. Lifestyle choices, including diet, exercise habits, and stress management, also evolve with age and can profoundly influence these metabolic markers. Hormonal shifts that occur as individuals age may further contribute to these alterations. Additionally, the combined effects of health conditions, medications, genetics, and dietary habits that accumulate over time can contribute to these patterns. Common age-related conditions like diabetes and heart disease could influence these metabolic trends across different age groups. Understanding these diverse factors is critical to grasping how age influences these crucial aspects of metabolic health, highlighted in the study findings. The analysis examining the correlation between HbA1c and key metabolic parameters uncovered a notable positive association with BMI within the age groups spanning 20-40 and 41-60 years, exhibiting coefficients of 0.53 (p<0.001) and 0.4 (p<0.001), respectively. (Figure 1). This association suggests that as HbA1c levels increase, so does BMI within these age groups. Similarly, in the same age ranges of 20-40 and 41-60, HbA1c demonstrated positive correlations with TC (r=0.17, p<0.001 and r=0.28, p=0.0006) and TG (r=0.2, p<0.001 and r=0.2, p=0.0151) (as shown in Figure 1). Moreover, in the age group of 61-80, a direct correlation was observed between HbA1c and LDL-C (Figure 6). All are summarised in Table 5. These findings suggest that metabolic parameters such as BMI, TC, TG, and LDL-C may be interlinked with HbA1c levels, potentially reflecting underlying metabolic processes and highlighting the intricate relationships between these variables, particularly in different age groups.

BMI

Based on the BMI categories (18.5-22.9, 23-24.9, ≥25), subgroup analyses revealed a distinct trend: as age increased, the prevalence of obesity increased. Concurrently, a marked increase in the incidence of diabetes was noted with higher levels of obesity. Additionally, individuals identified as obese exhibited a notably higher prevalence of hypertriglyceridemia (refer to Table 6 for the results). The outcomes delineated a statistically significant differentiation among diverse BMI groups concerning TG (p=0.0002), Age (p<0.0001), and HbA1c (p<0.0001) (shown in Figure 3). Mean TG values displayed an escalating trend from 1.9±1.1 to 2.0±1 and 2.4±1.5 with increasing BMI. A parallel pattern was observed in Age (43±9.9, 44±8.7, 56±6.9) and HbA1c (5.4±1.5, 5.3±1.4, 9±2.2) (refer to Table 6). However, no significant variances were detected in HDL-C (p=0.6516), LDL-C (p=0.0627), and TC (p=0.7078). The correlation analysis between HbA1c and other parameters unveiled a positive correlation with TC across three BMI groups (r=0.29, p=0.0053, r=0.22, p=0.0279, and r=0.19, p<0.0001) (depicted in Figure 4). Similar relationships were observed with TG (r=0.23, p=0.0234 and r=0.18, p<0.0001) (Figure 4) in both normal-weight and obese cohorts. Furthermore, a direct correlation was observed between HbA1c and age, specifically within the obese group. It is noteworthy that no significant differences were observed in HDL-C and LDL-C.

Discussion

The linkage between diabetes and cardiovascular disease (CVD) has long been established and substantiated through historical evidence. Previous studies have suggested that HbA1c is a marker for glycemic control and a reliable predictor of lipid profile due to its interconnected nature. Nonetheless, various factors contribute to discrepancies in the results. This study explored the relationship between HbA1c and lipid profile within the 1000 subjects, where 52.3% exhibited dyslipidemia and 76.1% had diabetes. Notably, 38% of patients displayed hypercholesterolemia, 62.5% had hypertriglyceridemia, 52.8% manifested low HDL-C levels, and 23.5% exhibited high LDL-C levels, recognised as cardiovascular risk factors.

Our findings elucidate a significant positive correlation between HbA1c, TC, and TG, aligning with similar conclusions documented in previous studies[14]. This study underscores that HbA1c directly indicates elevated TG and TC levels, consequently aiding in assessing diverse vascular complications. Moreover, HbA1c notably correlated with age and BMI, emphasising its utility as a clinical marker.

Nevertheless, our study failed to establish significant associations between HbA1c and HDL-C or LDL-C levels. These results deviate from a study by Khan et al.[15], which reported a negative correlation with HDL-C and a positive one with LDL-C. It is worth noting that other research has also indicated an absence of correlation between HbA1c and HDL-C. This discrepancy might be attributed to the slightly higher HbA1c levels observed in the female group compared to the male group, as females typically exhibit elevated HDL-C levels compared to males[16], thereby precluding a significant negative correlation. Subgroup analyses stratified by gender offered insights into this disparity.

Subgroup Diabetes and non-diabetes

Numerous studies have elucidated the intricate interplay between diabetes and dyslipidemia. Within this current study, demographic profiles of individuals with diabetes revealed a notably elevated prevalence of obesity (96%) as well as higher incidences of hypercholesterolemia (41% compared to 29% in non-diabetic individuals) and hypertriglyceridemia (68% compared to 44% in non-diabetic individuals). Moreover, subjects exhibiting HbA1c levels equal to or exceeding 6.5% displayed significantly elevated triglyceride (TG) and total cholesterol (TC) levels compared to those with HbA1c levels below 6.5%.

Furthermore, HbA1c exhibited a direct and significant positive correlation with TC and TG within the diabetic group, findings that are consistent with previous research highlighting the reciprocal between diabetes and dyslipidemia, wherein each condition exacerbates the other, consequently heightening the risk of cardiovascular disease [17]. This phenomenon can be attributed to chronic hyperglycemia inducing apolipoprotein glycation and disrupting normal lipoprotein metabolism pathways. Inadequate insulin secretion or function, coupled with delayed removal of TG-rich lipoproteins, leads to increased TG levels. Augmented liver synthesis and release of TG due to enhanced raw material supply contribute to elevated TG and TC levels[27]. Furthermore, a lipotoxic mechanism facilitated by triglycerides can impede gastric or neural pathways that regulate glycemic control[18].

It can be inferred that HbA1c is a reasonable predictor for dyslipidemia in diabetes by forecasting TC and TG status, thereby identifying patients at heightened cardiovascular risk. Conversely, an HbA1c value below 6.5% appears conducive to mitigating cardiovascular risk owing to a normalised lipid profile. Nonetheless, no significant disparities were observed in low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels. These findings diverge from prior

studies that reported significantly elevated TC, LDL-C, and TG levels alongside reduced HDL-C in diabetic individuals compared to their non-diabetic counterparts. These discrepancies may be ascribed to varying study designs and the selection of study populations, suggesting that factors beyond diabetes could contribute to dyslipidemia development[19]. Notably, this study identified significant positive correlations between age and BMI in individuals with diabetes, potentially representing pertinent contributing factors.

However, our study revealed a higher prevalence of hypercholesterolemia in males (65%) compared to females (59%) within the study population. This discrepancy in outcomes could be attributed to differences in BMI distributions. Specifically, male participants exhibited an obesity rate of 84% (BMI > 25)[20], potentially influencing the lipid profiles observed between the genders.

Subgroup gender

Gender-based analysis revealed significantly higher BMI, total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) values in females than in males. This observation aligns with findings from several other studies, except HbA1c and low-density lipoprotein cholesterol (LDL-C), where no significant differences between genders were noted. The disparity in gender-specific profiles may stem from variations in sex hormone levels affecting body fat distribution and the impact of estrogen post-menopause, which regulates glucose metabolism, inhibits lipid accumulation, and reduces inflammation [21]. Notably, within the age range of 41-60, 316 females were included in the study.

Subgroup age

Age exhibits a notable correlation with dyslipidemia, a relationship well-documented in numerous studies that highlight an increasing prevalence of dyslipidemia with advancing age. Within our investigations, elderly individuals demonstrated a significant association with elevated triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C), displaying a positive correlation with HbA1c levels. Additionally, subjects with higher HbA1c values tended to be older than those with lower HbA1c levels, thereby linking age with diabetes[22]. In both male and female cohorts, the most pronounced differences in body mass index (BMI) were observed at younger ages, with a strong positive correlation between BMI and HbA1c evident in older age groups. These findings bear similarities to previous research indicating that increasing age and BMI elevate the risk of developing diabetes.

Subgroup BMI

In our study, the average BMI of our participants exceeded 30, signifying obesity within the cohort. Obesity assumes a pivotal role in the pathophysiology of diabetes, as evidenced by a notable rise in the incidence of diabetes and the mean HbA1c levels among individuals with a BMI exceeding 25. Prior research has highlighted the link between obesity, physical inactivity, and compromised blood sugar regulation. This association is attributed to the metabolic strain imposed by increased BMI, which hampers HDL-C production, exacerbates pancreatic cell dysfunction, and fosters insulin resistance[23].

Clinical Implications

HbA1c serves as a metric for glycemic regulation and a predictor of dyslipidemia in individuals with diabetes. Poorly controlled diabetic patients often exhibit elevated lipid levels. Our study underscores the critical role of glycemic management in addressing dyslipidemia. Within our patient cohort, insufficient glycemic control, defined by an HbA1c level of ≥6.5%, was associated with heightened triglyceride (TG) and total cholesterol (TC) levels. Per recommendations

from the American Diabetes Association, regular monitoring of serum lipids in diabetic patients is crucial due to the heightened risk of dyslipidemia[26]. Elevated HbA1c levels have been identified as a significant risk factor for cardiovascular ailments such as coronary artery disease and mortality[20]. Therefore, our findings emphasise the importance of effective glycemic control in managing diabetic complications, dyslipidemia, and the risk of cardiovascular diseases. This relationship was further corroborated by consistent results in subgroup analyses stratified by sex, age, and BMI, with no apparent correlation between HbA1c and high-density lipoprotein cholesterol (HDL-C) across different groups. Age and BMI are intricately linked to diabetes and obesity. Dietary choices significantly impact patients' glucose and lipid profiles, particularly HDL-C levels, where elevated circulating insulin levels are associated with reduced HDL-C levels[17]. Increased intake of saturated fatty acids tends to elevate lipid profiles, while polyunsaturated fatty acids have the opposite effect, reducing elevated triglycerides.

The mechanisms underlying the deterioration of blood glucose regulation and dyslipidemia, leading to atherosclerosis, are multifaceted. Hyperglycemia triggers inflammatory responses and mitochondrial oxidative stress, directly contributing to vascular endothelial dysfunction. Reduced HDL-C levels trigger local inflammation, endothelial thrombosis, and endothelial cell apoptosis and impede vascular repair. Inflammation-induced by hyperglycemia and low HDL-C disrupts the immune balance, leading to immune system dysregulation. The dual impact of rising HbA1c levels and declining HDL-C levels accelerates atherosclerosis progression. This underscores the significance of comprehensive management of glucose-metabolic disorders and dyslipidemia in averting atherosclerosis[16].

Limitations and Future Research Directions

One limitation of this study is the absence of background patient data, including details such as diabetes type, blood pressure, smoking habits, dietary patterns, physical activity levels, and medication usage — factors widely recognised as risk determinants for cardiovascular disease. The lack of comprehensive information restricts our ability to assess these variables' contributions to dyslipidemia. The study did not stratify data based on treatment modalities, potentially influencing the study outcomes. On the positive side, the study's strength lies in the comprehensive biochemical profiles available for the patients, enabling thorough comparisons and correlations. Dietary habits and physical activity levels are known to vary by ethnicity, with lower diabetes prevalence observed among Asian and Black populations compared to White individuals[11,24].). Exploring the relationship between HbA1c levels and lipid profiles across different ethnic groups could yield valuable insights. Furthermore, considering the two distinct types of diabetes—Type 1, which is inherited, and the other acquired form—it is crucial to acknowledge that dyslipidemia can also have genetic underpinnings passed down from parents. Future research directions might delve into molecular investigations, focusing on specific genes associated with a heightened risk of developing diabetes and dyslipidemia[25].

Conclusions

The findings of this investigation reveal a noteworthy association between HbA1C levels and various circulating lipid parameters. Notably, we observed a substantial contrast in the lipid profiles between two cohorts categorised by their HbA1C levels (indicating excellent and poor glycemic control). This underscores the dual role of HbA1c as a dependable marker for glycemic regulation and a valuable indicator for anticipating dyslipidemia in individuals with diabetes. Consequently, utilising the glycemic parameter could facilitate the early

detection of dyslipidemia. Therefore, consistent monitoring of blood glucose levels, HbA1C values, and lipid profiles is strongly advised as part of routine health assessments.

Declaration of competing interest
The authors declare that they have no competing interests.

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References

1. Manemann SM, Bielinski SJ, Moser ED, St. Sauver JL, Takahashi PY, Roger VL, Olson JE, Chamberlain AM, Remaley AT, Decker PA, Killian JM, Larson NB. Variability in Lipid Levels and Risk for Cardiovascular Disease: An Electronic Health Record–Based Population Cohort Study. J Am Heart Assoc. 2023;12(5). doi:10.1161/JAHA.122.027639.
2. Moumen A, Zraidi M, Derrou S, Benabdelfedil Y, El Guendouz F. Management of Cardiovascular Risk among Moroccan Patients with Type 2 Diabetes: A Cross-Sectional Study. Clin Diabetol. 2023;12(3):171-8. doi:10.5603/DK.a2023.0021.
3. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomark Insights. 2016;11:95-104. doi:10.4137/Bmi.s38440.
4. Lind M, Odén A, Fahlén M, Eliasson B. A systematic review of HbA1c variables used in the study of diabetic complications. Diabetes Metab Syndr Clin Res Rev. 2008;2(4):282-93. doi:10.1016/j.dsx.2008.04.006.
5. Chhatriwala MN, Patel MP, Patel DS, Shah HN. Relationship between Dyslipidemia and Glycemic Status in Type-2 Diabetes Mellitus. Natl J Lab Med. 2019. doi:10.7860/njlm/2019/42887:2371.
6. Al-Shaheeb S, Hashim HK, Mohammed AK, Almashhadani HA, Al Fandi A. Assessment of lipid profile with HbA1c in type 2 diabetic Iraqi patients. Bionatura. 2022;7(3). doi:10.21931/RB/2022.07.03.29.
7. Sharahili AY, et. al. Correlation of HbA1c Level with Lipid Profile in Type 2 Diabetes Mellitus Patients Visiting a Primary Healthcare Center in Jeddah City, Saudi Arabia: A Retrospective Cross-Sectional Study. Diseases. 2023;11(4):154. doi: 10.3390/diseases11040154
8. Made Junior Rina Artha I, Bhargah A, Dharmawan NK, Pande UW, Triyana KA, Mahariski PA, Yuwono J, Bhargah V, Putu Yuda Prabawa I, Manuaba IBA P, Ketut Rina I. High level of individual lipid profile and lipid ratio as a predictive marker of poor glycemic control in type-2 diabetes mellitus. Vasc Health Risk

Manag. 2019a;15:149-57. doi:10.2147/VHRM.S209830.

9. Wang S, Ji X, Zhang Z, Xue F. Relationship between lipid profiles and glycemic control among patients with type 2 diabetes in Qingdao, China. Int J Environ Res Public Health. 2020;17(15):1-11. doi:10.3390/ijerph17155317.

10. Kumar S, Kumari B, Kaushik A, Banerjee A, Mahto M, Bansal A. Relation Between HbA1c and Lipid Profile Among Prediabetics, Diabetics, and Non-diabetics: A Hospital-Based Cross-Sectional Analysis. Cureus. 2022. doi:10.7759/cureus.32909.

11. Malathi RD, Fatima A, Ahmed MI. Evaluation of Dyslipidemia with HbA1c levels in Diabetes Mellitus. Int J Clin Biochem Res. 2022;9(4):306-10. doi:10.18231/j.ijcbr.2022.060.

12. Held C, Hadziosmanovic N, Aylward PE, Hagström E, Hochman JS, Stewart RA, White HD, Wallentin L. Body Mass Index and Association With Cardiovascular Outcomes in Patients With Stable Coronary Heart Disease – A STABILITY Substudy. J Am Heart Assoc. 2022;11(3). doi:10.1161/JAHA.121.023667.

13. Tatman R. R vs Python: The Kitchen Gadget Test, Version 1. Kaggle; 2017 [cited 2017 Dec 20]. Available from: https://www.kaggle.com/rtatman/r-vs-python-the-kitchen-gadget-test.

14. Lin YC, Tu HP, Wang TN. Blood lipid profile, HbA1c, fasting glucose, and diabetes: a cohort study and a two-sample Mendelian randomisation analysis. J Endocrinol Invest. 2024;47(4):913-25. doi:10.1007/s40618-023-02209-x.

15. Khan HA, Sobki SH, Khan SA. Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidaemia. Clin Exp Med. 2007;7(1):24-9. doi:10.1007/s10238-007-0121-3.

16. Hu X, Li W, Wang C, Zhang H, Lu H, Li G, Zhou Y, Dong H. Association between the Plasma-Glycosylated Hemoglobin A1c/High-Density Lipoprotein Cholesterol Ratio and Carotid Atherosclerosis: A Retrospective Study. J Diabetes Res. 2021;2021. doi:10.1155/2021/9238566.

17. Samdani TS, Mitra P, Rahim MA. Relationship of Glycated Haemoglobin with Lipid Profile among Patients with Type 2 Diabetes Mellitus. BIRDEM Med J. 2017;7(1):43-7. doi:10.3329/birdem.v7i1.31271.

18. Singh Bal B, Kumar Salwan S, Chandarana U. Study of Association between HbA1c Level and Lipid Profile in Type 2 Diabetes Mellitus. Ann Int Med Dent Res. 2019;3.

19. Gedikli MA, Sarı Kalın B, Aktaş A. Relationship Between HbA1c Level and Triglyceride/HDL Cholesterol Ratio and Triglyceride Glucose Index in Diabetes Patients. Bagcilar Med Bull. 2022;7(1):27-31. doi:10.4274/bmb.galenos.2022.2021-09-095.

20. Eeg-Olofsson K, Zethelius B, Gudbjörnsdottir S, Eliasson B,

Svensson AM, Cederholm J. Considerably decreased risk of cardiovascular disease with combined reductions in HbA1c, blood pressure and blood lipids in type 2 diabetes: Report from the Swedish National Diabetes Register. Diabetes Vasc Dis Res. 2016;13(4):268-77. doi:10.1177/1479164116637311.

21. Casagrande SS, Fradkin JE, Saydah SH, Rust KF, Cowie CC. The prevalence of meeting A1C, blood pressure, and LDL goals among people with diabetes, 1988-2010. Diabetes Care. 2013;36(8):2271-9. doi:10.2337/dc12-2258.

22. Kidwai SS, Nageen A, Bashir F, Ara J. HbA1c – a predictor of dyslipidemia in type 2 diabetes mellitus. Pak J Med Sci. 2020;36(6):1339-43. doi:10.12669/pjms.36.6.2000.

23. Made Junior Rina Artha I, Bhargah A, Dharmawan NK, Pande UW, Triyana KA, Mahariski PA, Yuwono J, Bhargah V, Putu Yuda Prabawa I, Manuaba IBA P, Ketut Rina I. High level of individual lipid profile and lipid ratio as a predictive marker of poor glycemic control in type-2 diabetes mellitus. Vasc Health Risk Manag. 2019b;15:149-57. doi:10.2147/VHRM.S209830.

24. Jia X, Hou Y, Xu M, Zhao Z, Xuan L, Wang T, Li M, Xu Y, Lu J, Bi Y, Wang W, Chen Y. Mendelian Randomization Analysis Support Causal Associations of HbA1c with Circulating Triglyceride, Total and Low-density Lipoprotein Cholesterol in a Chinese Population. Sci Rep. 2019;9(1). doi:10.1038/s41598-019-41076-6.

25. Zheng D, Dou J, Liu G, Pan Y, Yan Y, Liu F, Gaisano HY, Lu J, He Y. Association between triglyceride level and glycemic control among insulin-treated patients with type 2 diabetes. J Clin Endocrinol Metab. 2019;104(4):1211-20. doi:10.1210/jc.2018-01656.

26. Čermák T, Laštovička P, Mužáková V, Líbalová M, Koukalová L, Kanďár R, Čegan A. Association of fatty acid profile in plasma lipid fractions with HbA1c in type 2 diabetic patients. Int J Diabetes Dev Ctries. 2016;36(1):23-33. doi:10.1007/s13410-015-0399-8.

27. Selvin E, Coresh J, Golden SH, Brancati FL, Folsom AR, Steffes MW. Glycemic Control and Coronary Heart Disease Risk in Persons With and Without Diabetes: The Atherosclerosis Risk in Communities Study.

Table 1: Demographic and clinical details of the patients

Parameters	Categories	N (%)	Mean	SD	Median	Range
Age (Years)	20-40	101 (10.1%)	54	8.8	55	20-79
	41-60	751 (75.1%)				
	60-80	148 (14.8%)				
BMI	Under-weight	0 (0%)	30	5	30	19-48
	Normal-weight	93 (9.3%)				
	Over-weight	97 (9.7%)				
	Obesity	810 (81%)				
HbA1c (%)	Non-Diabetes	239 (23.9%)	8.3	2.5	8	0.9-15
	Diabetes	761 (76.1%)				
TC (mmol/L)	Normal (≤5.18)	620 (62%)	4.9	1.3	4.8	0-10
	High (>5.18)	380 (38%)				
TG (mmol/L)	Normal (≤1.7)	375 (37.5%)	2.3	1.4	2	0.3-14
	High (>1.7)	625 (62.5%)				
HDL-C (mmol/L)	Normal (≥1.04)	462(46.2%)	1.2	0.66	1.1	0.2-9.9
	Low (< 1.04)	528(52.8%)				
LDL-C (mmol/L)	Normal (≤3.37)	765(76.5%)	2.6	1.1	2.5	0.3-9.9
	High (>3.37)	235(23.5%)				

Table 2 Correlation between Age, BMI, HbA1c, and lipid parameters

Parameter	AGE		HbA1c		BMI	
	Correlation	p-value	Correlation	p-value	Correlation	p-value
AGE	1.00	-	0.38**	<0.001	0.38**	<0.001
HbA1c	0.38	0.247	1.00	-	0.41**	<0.001
TC	0.04**	<0.001	0.18**	<0.001	0.01	0.666
TG	0.15	0.527	0.22**	<0.001	0.11**	<0.001
HDL-C	-0.02	0.611	0.03	0.361	0.07*	0.022
LDL-C	0.02**	<0.001	0.01	0.727	-0.07*	0.033
BMI	0.38**	<0.001	0.41**	<0.001	1.00	-

Parameter	TC		TG		HDL-C		LDL-C	
	Correlation	p-value	Correlation	p-value	Correlation	p-value	Correlation	p-value
AGE	0.04	0.247	0.15**	<0.001	-0.02	0.527	0.02	0.611
HbA1c	0.18**	<0.001	0.22**	<0.001	0.03	0.361	0.01	0.727
TC	1.00	-	0.32**	<0.001	0.10**	<0.001	0.42**	<0.001
TG	0.32**	<0.001	1.00	-	-0.08	0.009	0.02	0.627
HDL-C	0.10**	0.001	-0.08*	0.009	1.00	-	-0.14**	<0.001
LDL-C	0.42**	<0.001	0.02	0.627	-0.14**	<0.001	1.00	-
BMI	0.01	0.666	0.11**	<0.001	0.07	0.022	-0.07*	0.033

**Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

Table 3: The bio-clinical parameter results of patients with type 2 diabetes mellitus, with and without complications.

Parameters	Categories	HbA1c %<6.5 (non-Diabetes)		HbA1c%≥6.5 (Diabetes)		p-value
		N	(%)	N	(%)	
Age (Years)	20-40	63	26%	38	5%	<0.0001*
	41-60	165	69%	586	77%	
	60-80	11	5%	137	18%	
BMI	Under-weight	0	0%	0	0%	<0.0001*
	Normal-weight	74	31%	19	2%	
	Over-weight	85	36%	12	2%	
	Obesity	80	33%	730	96%	
TC (mmol/L)	Normal (≤5.18)	169	71%	451	59%	<0.0001*
	High (>5.18)	70	29%	310	41%	
TG (mmol/L)	Normal (≤1.7)	135	56%	65	9%	<0.0001*
	High (>1.7)	104	44%	521	68%	
HDL-C (mmol/L)	Normal (≥1.04)	130	54%	408	54%	0.7208
	Low (< 1.04)	109	46%	353	46%	
LDL-C (mmol/L)	Normal (≤3.37)	182	76%	583	77%	0.6352
	High (>3.37)	57	24%	178	23%	

*Statistically significant

Table 4: Distribution on comparison of HbA1c, BMI, and lipid profile categorized by age groups

Parameters	Categories	Age:20-40		Age:41-60		Age:61-80		p-value
		N	(%)	N	(%)	N	(%)	
BMI	Under-weight	0	0%	0	0%	0	0%	<0.0001*
	Normal-weight	36	36%	53	7%	4	3%	
	Over-weight	30	30%	66	9%	1	1%	
	Obesity	35	35%	632	84%	143	97%	
HbA1c (%)	Non-Diabetes	66	65%	165	22%	11	7%	<0.0001*
	Diabetes	35	35%	586	78%	137	93%	
TC (mmol/L)	Normal (≤5.18)	69	68%	462	62%	89	60%	0.2186
	High (>5.18)	32	32%	289	38%	59	40%	
TG (mmol/L)	Normal (≤1.7)	20	20%	275	37%	50	34%	<0.0001*
	High (>1.7)	51	50%	476	63%	98	66%	
HDL-C (mmol/L)	Normal (≥1.04)	58	57%	405	54%	75	51%	0.4493
	Low (< 1.04)	43	43%	346	46%	73	49%	
LDL-C (mmol/L)	Normal (≤3.37)	76	75%	581	77%	108	73%	0.0164*
	High (>3.37)	25	25%	170	23%	40	27%	

*Statistically significant

Table 5: Correlations between HbA1c, BMI, and lipid profile panel categorized by age groups

Age:20-40	HbA1c vs. TC	HbA1c vs. TG	HbA1c vs. HDL	HbA1c vs. LDL	HbA1c vs. BMI
Pearson r	-0.062	0.077	-0.12	0.048	0.53
95% confidence interval	-0.25 to 0.13	-0.12 to 0.27	-0.31 to 0.076	-0.15 to 0.24	0.38 to 0.66
R squared	0.0039	0.0059	0.015	0.0023	0.28
P value	0.535	0.4441	0.2263	0.6318	<0.0001*

Age:41-60	HbA1c vs. TC	HbA1c vs. TG	HbA1c vs. HDL	HbA1c vs. LDL	HbA1c vs. BMI
Pearson r	0.17	0.2	0.044	-0.028	0.4
95% confidence interval	0.10 to 0.24	0.13 to 0.27	-0.027 to 0.12	-0.10 to 0.043	0.33 to 0.45
R squared	0.029	0.04	0.002	0.00081	0.16
P value	<0.0001*	<0.0001*	0.2263	0.4368	<0.0001*

Age:61-80	HbA1c vs. TC	HbA1c vs. TG	HbA1c vs. HDL	HbA1c vs. LDL	HbA1c vs. BMI
Pearson r	0.28	0.2	-0.015	0.19	0.041
95% confidence interval	0.12 to 0.42	0.039 to 0.35	-0.18 to 0.15	0.031 to 0.34	-0.12 to 0.20
R squared	0.078	0.04	0.00023	0.037	0.0017
P value	0.0006*	0.0151*	0.8549	0.02	0.6168

*Statistically significant

Table 6: Distribution on comparison of Age group, HbA1c, and lipid profile categorized by BMI

Parameters	Categories	BMI18.5-22.9		BMI23-24.9		BMI≥25		p-value
		N	(%)	N	(%)	N	(%)	
Age (Years)	20-40	36	39%	30	31%	35	4%	<0.0001*
	41-60	53	57%	66	68%	632	78%	
	60-80	4	4%	1	1%	143	18%	
HbA1c (%)	Non-Diabetes	74	80%	85	88%	80	10%	<0.0001*
	Diabetes	19	20%	12	12%	730	90%	
TC (mmol/L)	Normal (≤5.18)	59	63%	61	63%	500	62%	0.7078
	High (>5.18)	34	37%	36	37%	310	38%	
TG (mmol/L)	Normal (≤1.7)	55	59%	45	46%	275	34%	0.0002*
	High (>1.7)	38	41%	52	54%	535	66%	
HDL-C (mmol/L)	Normal (≥1.04)	54	58%	55	57%	429	53%	0.6516
	Low (< 1.04)	39	42%	42	43%	381	47%	
LDL-C (mmol/L)	Normal (≤3.37)	68	73%	66	68%	631	78%	0.0627
	High (>3.37)	25	27%	31	32%	179	22%	

*Statistically significant

Table 7: Correlations between HbA1c, Age, and lipid profile panel categorized by BMI

BMI18.5-22.9	HbA1c vs. TC	HbA1c vs. TG	HbA1c vs. HDL-C	HbA1c vs. LDL-C	HbA1c vs. AGE
Pearson r	0.29	0.23	-0.022	0.14	-0.0058
95% confidence interval	0.089 to 0.46	0.033 to 0.42	-0.22 to 0.18	-0.064 to 0.34	-0.21 to 0.20
R squared	0.082	0.055	0.00046	0.02	0.000034
P value	0.0053*	0.0234*	0.8376	0.177	0.9559
BMI23-24.9	HbA1c vs. TC	HbA1c vs. TG	HbA1c vs. HDL-C	HbA1c vs. LDL-C	HbA1c vs. AGE
Pearson r	0.22	0.011	-0.019	-0.034	-0.17
95% confidence interval	0.025 to 0.40	-0.19 to 0.21	-0.22 to 0.18	-0.23 to 0.17	-0.36 to 0.030
R squared	0.05	0.00011	0.00037	0.0011	0.029
P value	0.0279*	0.9179	0.8519	0.7421	0.0955

BMI>25	HbA1c vs. TC	HbA1c vs. TG	HbA1c vs. HDL-C	HbA1c vs. LDL-C	HbA1c vs. AGE
Pearson r	0.19	0.18	0.042	0.067	0.16
95% confidence interval	0.12 to 0.26	0.12 to 0.25	-0.027 to 0.11	-0.0020 to 0.14	0.087 to 0.22
R squared	0.036	0.034	0.0017	0.0045	0.024
P value	<0.0001*	<0.0001*	0.2359	0.0571	<0.0001*

*Statistically significant

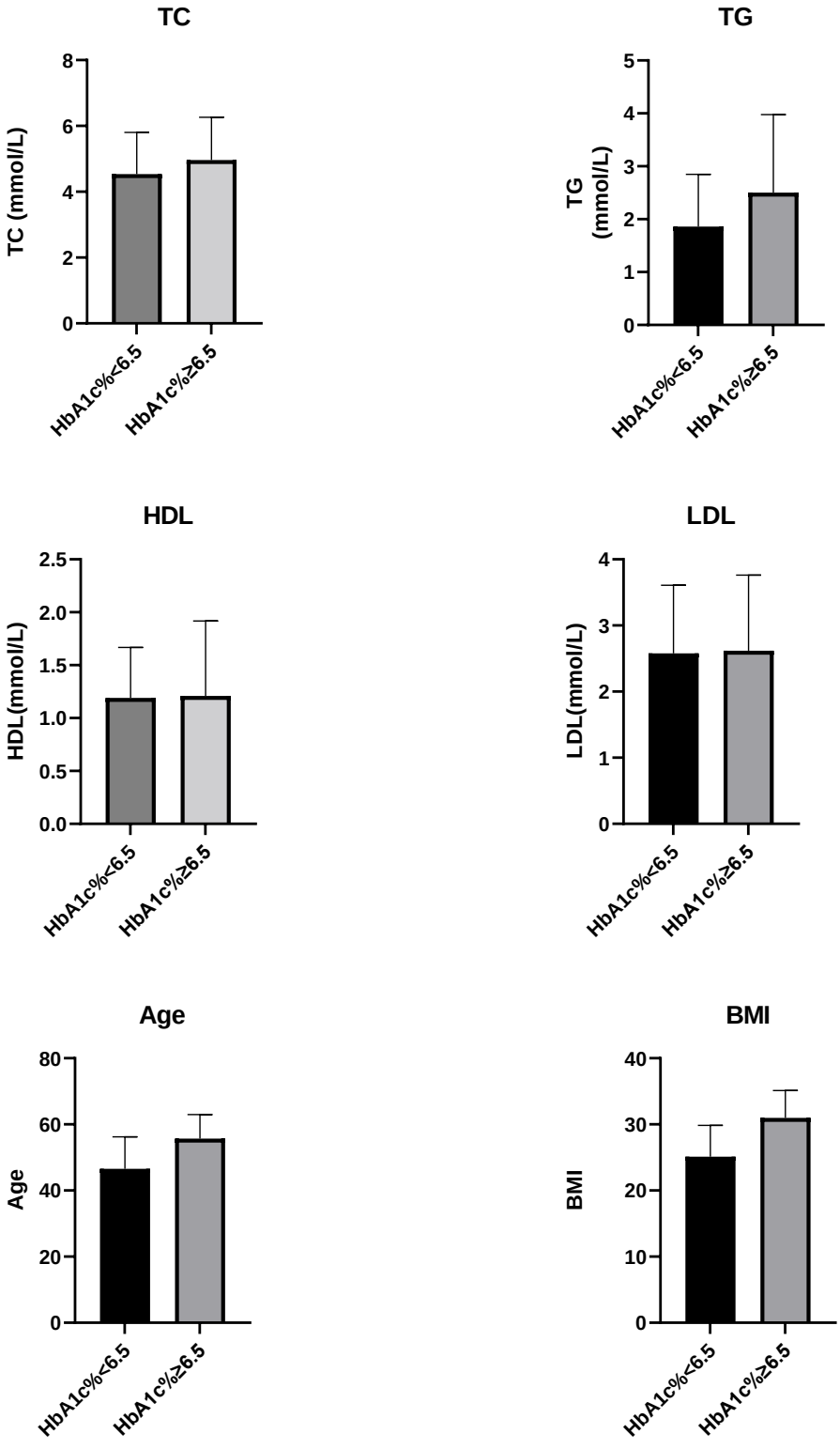

Figure 1 : Comparison between biochemical parameters between Diabetes and non-Diabetes

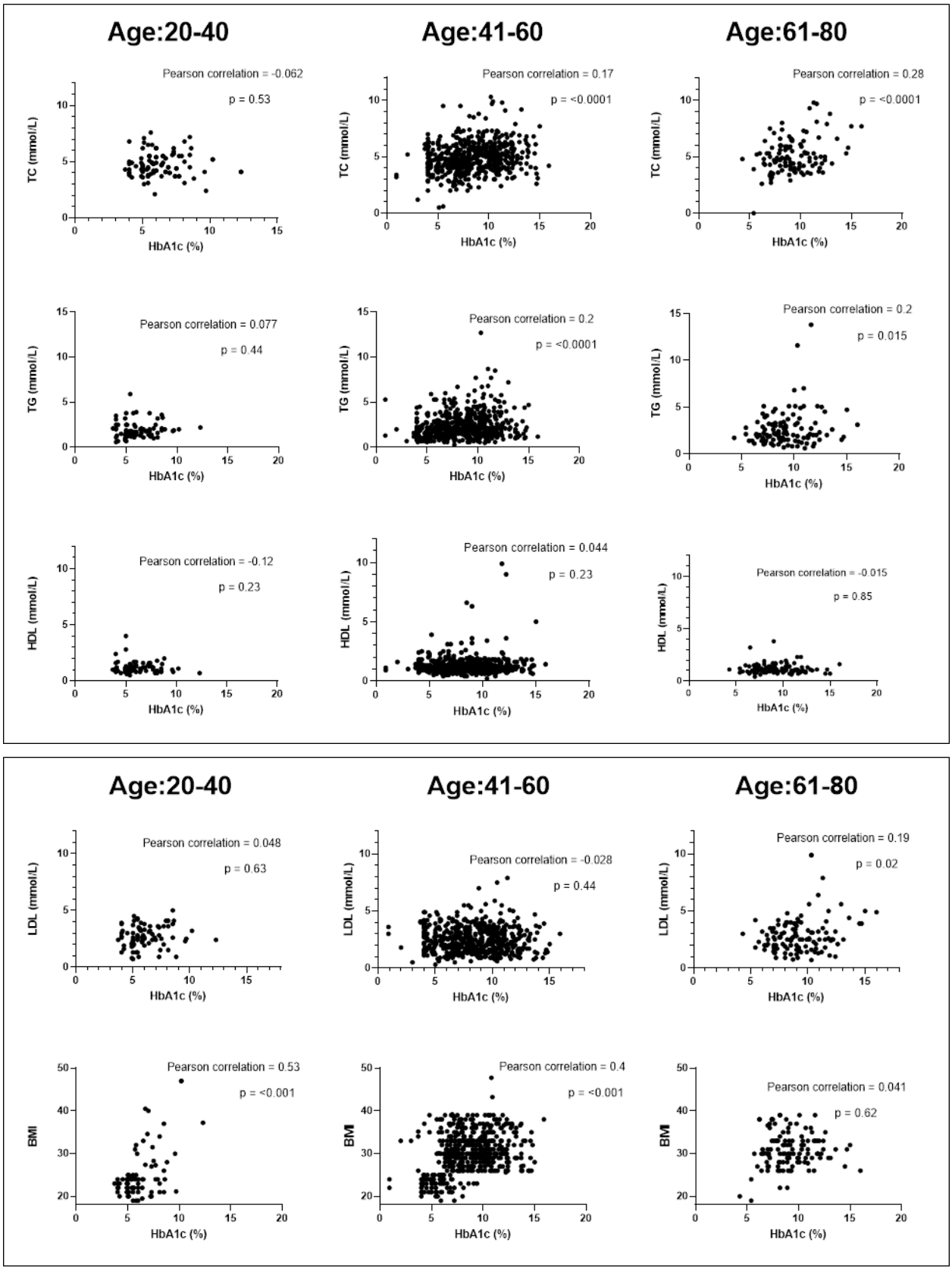

Figure 2. Correlations between HbA1c, BMI, and lipid profile panel categorized by age groups

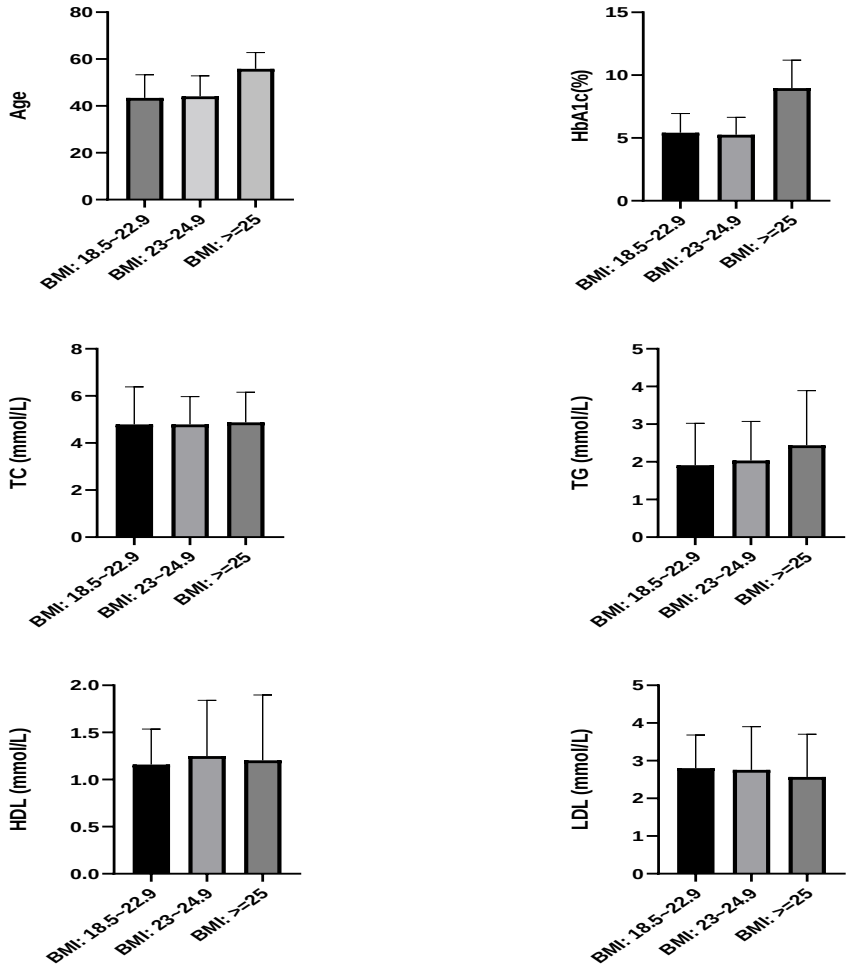

Figure 3: Comparison between biochemical parameters categorized by BMI

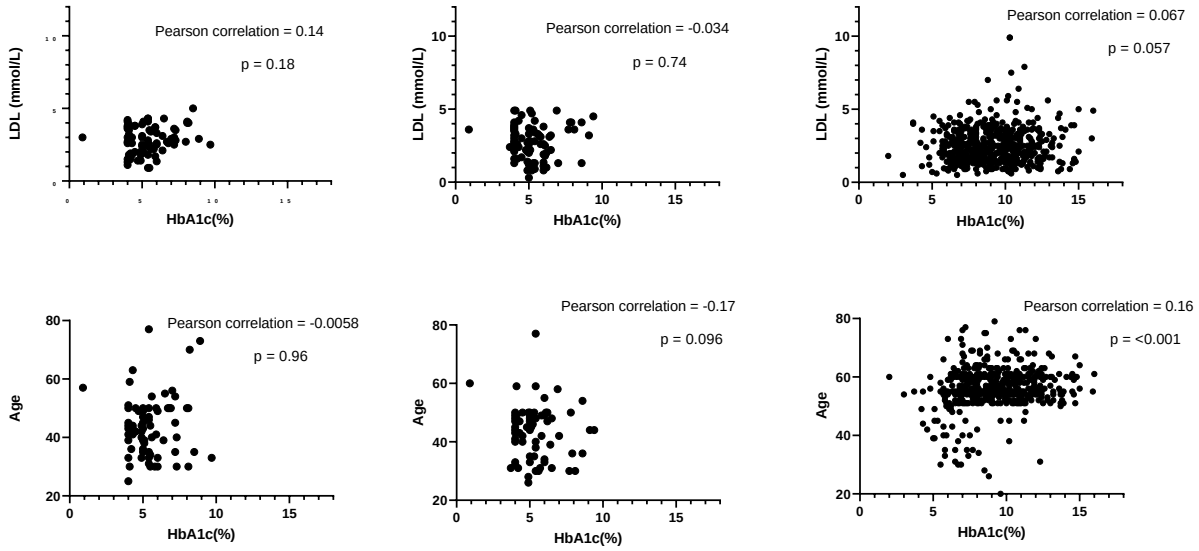

Figure 4. Correlations between HbA1c, Age, and lipid profile panel categorized by BMI

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