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Recommendations on measurement units – why and how

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On behalf of the IFCC-IUPAC Committee on Nomenclature for Properties and Units (C-NPU)

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ABSTRACT

Globally, laboratories are producing, communicating, and exchanging millions of laboratory examination values to multiple parties every day. For most values, 'measurement units' are required to make the numerical values comparable and meaningful. However, a non-systematic use of 'measurement units' can create errors in communication between health care providers and become a risk to patient safety. Therefore, the Committee of Nomenclature for Properties and Units (C-NPU) recommends using an unambiguous terminology of 'measurement units', for daily patient care and scientific publications. In this work, C-NPU summarizes the recommendations on 'measurement units', explaining the reasons and the principles of the 'measurement units' used in laboratory medicine.

INTRODUCTION

'Measurement unit' (unit) is a well-understood and necessary concept in laboratory medicine. Without units, most quantitative laboratory examination values will not make sense and are not comparable. Dybkær and Jørgensen wrote in 1967: "To state that the mass concentration of haemoglobin in a blood sample is 25 is essentially meaningless. If the unit g/L is assumed, the patient is considered anaemic. If the unit g/dL is assumed, the patient is considered to be polycytaemic" (1).

With the introduction of the International System of Units (SI units) (2) in the 1960's, the worldwide scientific laboratory societies have accepted and, to a large extent, implemented the SI units for presentation of laboratory reports in health care and research. However, as indicated by the recent campaign of the European Federation of Clinical Chemistry and laboratory Medicine (EFLM), there is nevertheless a further need of standardisation or harmonisation on a national, regional, and international level (3). The campaign recommended implementation of the "principles on units", proposed by Dybkær and Jørgensen in 1967 (1). These principles are more restricted than the original SI-system to ensure unambiguity in reporting, presenting, and exchanging quantity values in health care. Each laboratory may choose any relevant units for reporting laboratory examination values, but when multiple parties are involved in exchanging laboratory reports, the choice should be limited to the "principles on units". Arguably, the principles will reduce the risk of post-analytical errors, e.g. misunderstanding and misinterpretation of laboratory reports and errors in communication between different health care personnel and organisations.

The "principles on units" in laboratory medicine, as initially proposed by Dybkær and Jørgensen, have been implemented in the Nomenclature for Properties and Unit (NPU) terminology (4, 5).

In this letter, we summarise the IFCC's and IUPAC's Recommendations and Technical Reports on relevant principles and rules on units in laboratory medicine, and the reasons behind these principles.

KIND-OF-QUANTITY, QUANTITY, AND MEASUREMENT UNIT

In order to understand the concept 'measurement unit', it is necessary to see its close relation to the other essential metrological concepts 'kind-of-quantity' and 'quantity'. 'Mass', 'substance concentration', and 'volume fraction' are examples of 'kinds-of-quantity' that place system and any relevant component in a mathematical relation. E.g., 'substance concentration' is defined as "amount-of-substance of component B divided by volume of system 1" or:

$$\frac{\text{Amount-of-substance of component B}}{\text{Volume of system 1}}$$

On a more tangible level, the system and component can be specified further including a magnitude, e.g. :

$$\frac{\text{Amount-of-substance of sodium ion}}{\text{Volume of Mr. Smith's plasma}} = 140 \text{ mmol/L}$$

The latter example is a 'quantity', having the formal and metrological definition "property of phenomenon, body, or substance, where the property has a magnitude that can be expressed as a number and a reference" (6). The differences between both concepts are shown in Table 1.

In laboratory medicine, eight 'base kinds-of-quantity' exist as listed in Table 2 with their corresponding 'base units' and 'quantity dimensions' (5). The 'base kinds-of-quantity (e.g. 'amount-of-substance') can be combined in various ways, forming 'derived kinds-of-quantity', e.g. 'substance concentration'.

Table 1 Kind-of-quantity and quantity

Level	Concepts	Examples	
		Verbal expression	Mathematical expression
Abstract	kind-of-quantity	substance concentration	$\frac{\text{Amount-of-substance of component B}}{\text{Volume of system 1}}$
Measurable	quantity	substance concentration of sodium ion in Mr. Smith's plasma is 143 mmol/L at 2:30 p.m. on 2nd May 2018.	$\frac{\text{Amount-of-substance of sodium ion}}{\text{Volume of Mr. Smith's plasma}} = 143\ \text{mmol/L}$

In the example for 'quantity', 'plasma' is the 'system', 'sodium ion' is the 'component' and 'substance concentration' is the 'kind-of-quantity'. Also, there is a magnitude according to the definition of 'quantity', as compared with the example for 'kind-of-quantity' that does not have a magnitude.

To 'substance concentration', the corresponding compound unit can be, e.g., mmol/L. To a (base or derived) kind-of-quantity, several corresponding units are possible. Examples of corresponding units to 'substance concentration' are 'mol/L', 'mmol/L', 'µmol/L', 'nmol/L', etc. A comprehensive description of 'kinds-of-quantity' and 'measurement units' can be found in IFCC's and IUPAC's 'Silver Book' (5)–together with 'kind-of-nominal-property (related to 'nominal properties' which have no magnitude).

Reporting solely the numerical value and unit may not be sufficient information on the examination because the possible corresponding 'kind-of-quantity' to e.g., 'g/L', could be 'mass concentration' or mass density'. Moreover, in order for the clinicians to assess the values of laboratory examinations, especially laboratory examination reports from other laboratories, it is essential to provide information about the generic nature of the laboratory examinations. Thus, C-NPU recommends to report, systematically, the system, component, kind-of-quantity (or kind-of-nominal property) and, when relevant, the unit for a given laboratory examination.

GENERAL RULES FOR SI UNITS AND NON-SI UNITS

It is recommended to use units with unambiguous definitions, accepted by international scientific communities. Such units can be SI units and non-SI units.

1. Base SI units

The definitions, symbols, and magnitudes of SI units are traced to accepted international references (Table 2) (2).

Examples

"The metre is the length of the path travelled by light in vacuum during a time interval of 1/299 792 458 of a second" (2).

"The second is the duration of 9 192 631 770 periods of the radiation corresponding to the transition between the two hyperfine levels of the ground state of the caesium 133 atom" (2).

Table 2 Base kinds-of-quantity, corresponding base units, and dimensions

Base kind-of-quantity	Base unit		Dimension
Term	Term	Symbol	Symbol
length	metre	m	L
mass	kilogram	kg	M
time	second	s	T
electrical current	ampere	A	I
thermodynamic temperature	kelvin	K	Θ
amount-of-substance	mole	mol	N
luminous intensity	candela	cd	J
number of entities	one	1	1

A list of base kinds-of-quantity and their corresponding base units and dimensions from IFCC's and IUPAC's 'Silver Book' (5). Note: 'Number of entities' is not an SI base kind-of-quantity but is used as a base kind-of-quantity in laboratory medicine.

Note: From the year 2019, all seven SI base units will be defined in terms of constants. The practical use of the seven SI base units will not change (7).

2. Unit of a given magnitude should have only one expression

For a unit with a given magnitude, there are several possible expressions, e.g.:

$$\frac{\text{mmol}}{\text{L}} = \frac{\mu\text{mol}}{\text{mL}} = \frac{\text{nmol}}{\mu\text{L}} = \frac{\text{pmol}}{\text{nL}}$$

Such variety may cause errors in communication between health personnel and organisations.

To ensure unambiguity in reporting values, only one expression for a unit of a given magnitude should be used.

3. Multiples and submultiples of units

To present numerical values in the interval of 0.1–999 (8) and to make values with very large or very small numerical values readable, the units can be combined with SI prefixes, expressed as either SI prefix symbols or SI prefix factors (numerical values) (Table 3).

To avoid errors in communication with potential patient mistreatments as consequences, multiple combinations of SI prefixes should not be allowed. Thus, the following rules apply:

- One SI prefix per unit
- The SI prefix belongs to the numerator only

Only one SI prefix per unit should be used. Combinations of SI prefixes are to be avoided (Table 4).

Table 3 SI prefixes: factors, terms, and symbols

Factor	Term	Symbol	Factor	Term	Symbol
10^{1}	deca	da	10^{-1}	deci	d
10^{2}	hecto	h	10^{-2}	centi	c
10^{3}	kilo	k	10^{-3}	milli	m
10^{6}	mega	M	10^{-6}	micro	µ
10^{9}	giga	G	10^{-9}	nano	n
10^{12}	tera	T	10^{-12}	pico	p
10^{15}	peta	P	10^{-15}	femto	f
10^{18}	exa	E	10^{-18}	atto	a
10^{21}	zetta	Z	10^{-21}	zepto	z
10^{24}	yotta	Y	10^{-24}	yocto	y

SI prefix table from the SI Brochure: The International System of Units (SI) [8th edition, 2006; updated in 2014] (BIPM) (2).

Table 4 Examples of one SI prefix per unit

Unit	Unit symbol	Examples of deprecated unit symbols	Examination example with correct unit
Picogram	pg	µµg 10^{-6}×µg	The mass of haemoglobin per erythrocyte in Mr. Smith's blood is 31 pg.
Millimole per litre	mmol/L	µmol/mL	The substance concentration of sodium in Mr. Smith's plasma is 134 mmol/L.

An SI prefix in the denominator should be avoided in a compound unit (Table 5).

An exception is that 'kilogram' (and not 'gram') is the base SI unit for mass and therefore can be expressed in the denominator as 'kg'.

4. Units for kinds-of-quantity of Dimension One (dimensionless)

Kind-of-quantity of Dimension One (dimensionless) is a "quantity for which all the exponents of the factors corresponding to the base

quantities in its quantity dimension are zero" (6). The 'base kind-of-quantity', 'number of entities' and kinds-of-quantity with the same 'kind-of-quantity' (dimension) in the numerator and denominator, e.g. 'mass fraction'

$$\frac{\text{Mass of component B}}{\text{Mass of system 1}}$$

or 'substance ratio'

$$\frac{\textit{Amount-of-substance of component B}}{\textit{Amount-of-substance of component C}}$$

have the dimension one, according to the rules of algebra. The corresponding coherent units for these kinds-of-quantity are numerical values, e.g., 'one' or SI prefix factors. The specified 'kind-of-quantity' along with the corresponding unit in the laboratory report provide the full nature of the quantity measured.

For the 'kinds-of-quantity' of Dimension One with the corresponding unit 'one', the unit symbol is often omitted for the values of these types (Table 6).

Table 5 Examples of SI prefix in the numerator

Unit	Unit symbol	Examples of deprecated symbols	Examination example with correct unit
Micromole per litre	µmol/L	nmol/mL	The substance concentration of bilirubins in Mr. Smith's plasma is 8 µmol/L.
Millimole per kilogram	mmol/kg	µmol/g	The mass of calprotectin in Mr. Smith's faeces is 8 mmol/kg.

Table 6 Examples of the unit 'one' for kinds-of-quantity of Dimension One

Unit	Unit symbol	Examples of deprecated symbols	Examination example with correct unit
One	1	-	The number of cavities in Mr. Smith's teeth is 2.
		kg/kg mg/mg	The mass fraction of free prostata specific antigen of total prostata specific antigen in Mr. Smith's plasma is 0.14.
		mol/mol mmol/mmol	The substance fraction of methaemoglobin of haemoglobin in Mr. Smith's blood is 0.03.
		L/L µL/µL	The volume fraction of erythrocytes of Mr. Smith's blood is 0.42.
		s/s min/min	The time of tissue factor-induced coagulation in Mr. Smith's plasma divided by the time of tissue factor-induced coagulation in the certified reference material, IRP 67/40, is 1.0 (INR).

To express very small or very large values, the units should be expressed as SI prefixes, according to the rules of multiples and submultiples of units. To avoid confusion with unit symbols, SI prefix factors should be used, not the SI prefix symbols (Table 7).

Consequently, redundant units are avoided because the same unit 'one' or SI prefix factors can represent units of various dimensionless kind-of-quantities and different expressions of a unit of a given magnitude (Table 6 and Table 8).

Another issue to address is conversion of unit from 'one' to '%' for a kind-of-quantity of dimension 'one', e.g. erythrocyte volume fraction (EVF). EVF can be expressed with 'one' or '%' as units, whereas 'one' is usually omitted. Without the indication of unit, it may be tempted to convert from 'one' to '%'. Values of erythrocyte volume fraction (EVF) will be reported either as "0.42" or "42". Despite the small and simple conversion from 'one' to '%' the laboratory report with both type of results

Table 7 Examples of SI prefix factors as units for kinds-of-quantity of Dimension One

Unit	Unit symbol	Examples of deprecated symbols	Examination example with correct unit
Ten to the power of 6 per litre	10^6/L	M/L* M×1/L	The number concentration of lymphocytes in Mr. Smith's cerebrospinal fluid is 8×10^6/L.
Ten to the power of -3 per litre	10^{-3}/L	m/L** m×1/L	The number concentration of RNA from Human immunodeficiency virus 1 in Mr. Smith's plasma is 0×10^{-3}/L.

** 'M' is the SI prefix symbols for 'mega'; ** 'm' is the SI prefix symbols for 'milli'.*

Table 8 Examples of SI prefix factor representing various units

Unit	Unit symbol	Examples of deprecated symbols	Examination example with correct unit
Ten to the power of -3	10^{-3}	g/kg	The mass fraction of ethanol of Mr. Smith's blood is 0.5×10^{-3}.
		mmol/mol	The substance ratio of albumin/creatininium in Mr. Smith's urine is 25×10^{-3}. (The albumin value is adjusted to the amount-of-substance of creatininium in urine).
		$\frac{1\ reticulocyte}{1000\ erythrocytes}$	The number fraction of reticulocytes of erythrocytes in Mr. Smith's blood is 10×10^{-3}.

will cause confusion, if not interpreted by a conscious human mind.

5. Units for quantities of the same sort of system, sort of component(s), and kind-of-quantity should differ at least by a factor of one thousand

A laboratory examination of a quantity with a given sort of system, sort of component(s), and kind-of-quantity can be reported with different corresponding units, according to the choice of the local laboratories. To reduce misinterpretations that may occur when exchanging laboratory results between hospitals or when health personnel change hospitals, it is recommended that the laboratories use units that differ by at least a prefix factor of one thousand (10^3) for the same type of examination performed in 2 or more laboratories.

E.g. Laboratory A measures the substance concentration of epinephrine in plasma with the unit, 'µmol/L'. Laboratory B performs the same type of measurement but present the value with a unit that differs at least by a prefix factor of one thousand. In this case Laboratory B uses the unit, 'nmol/L'.

Example

NPU14042 Plasma—Epinephrine; substance concentration = ? µmol/L

NPU04625 Plasma—Epinephrine; substance concentration = ? nmol/L

This recommendation is to prevent overlapping intervals of value sets for a specific laboratory examination. Often, value sets vary for the same laboratory examination using different units, but these variations may overlap when the units differ by a factor of 10 or 100, e.g. 'cm' and 'mm', '%' and '‰', or 'dL' and 'L'. The overlaps can cause misinterpretation, when the clinicians incorrectly assume use of the unit they are familiar with for a result from another laboratory (see example below). Thus, the use of SI prefix factors: centi (c), deci (d), deca (da) and hecto (h) are discouraged, except when the units are lifted to a power (see section 7.3).

Example

Laboratories A and B (in Hospitals A and B, respectively) measure number fraction of the reticulocytes among erythrocytes in Mr. Smith's blood with the use of two different units. The units differ by a factor of 10 (see below laboratory reports from laboratories A and B).

The patient is regularly admitted to Hospital B, but due to practical difficulties, a blood sample from the patient is analysed by Laboratory A in the patient's hometown. The health care personnel at hospital B may not react adequately on the value '1' from laboratory A on 24th January, because the value lies in a familiar value set interval and could mistakenly be interpreted to be within Laboratory B's reference interval (Table 9).

Table 9 Example of a cumulative laboratory report from two different laboratories

Laboratory examination	12th Jan	20th Jan	24th Jan	Reference interval	Unit
Erythrocytes (Blood)—Reticulocytes; number fraction*	-	-	1	5–22	$\times 10^{-3}$
Erythrocytes (Blood)—Reticulocytes; number fraction**	1	0.8	-	0.5–2.2	$\times 10^{-2}$

** Examination result from Lab A.; ** Examination result from Lab B.*

6. Non-SI units

Besides the non-SI units accepted for use together with the SI system, e.g., litre, (Table 10), there are two important internationally used expressions for non-SI units in laboratory medicine: 'WHO International Unit' (IU) and '(procedure defined unit)' (p.d.u.).

6.1 WHO International Unit (IU)

The term 'WHO International Unit' (IU) does not indicate one unit but comprises a heterogeneous group of units, each defined by internationally certified reference material (CRM), (e.g. a WHO International Standard). Thus, the given CRM defines the material and magnitude of the

Table 10 Non-SI units accepted for use with the International System of Units

Term	Symbol
litre	L
tonne	t
day	d
hour	h
minute	min
Dalton	Da

An extract of a list of accepted non-SI units from BIPM (2).

Table 11 Examples of use of SI prefix for 'International Unit' and 'enzyme unit'

Unit	Unit symbol	Examples of deprecated symbols	Examination example with correct unit
10^3 International Unit per litre	$\times 10^3$ IU/L	kIU/L	The arbitrary substance concentration of Birch -IgE in Mr. Smith's plasma is 10×10^3/L.
10^{-3} International Unit per litre	$\times 10^{-3}$ IU/L	mIU/L	The arbitrary number concentration of RNA from Hepatitis C virus in Mr. Smith's plasma is 200×10^{-3} IU/L.
10^{-3} enzyme unit per litre	mU/L	$\times 10^{-3}$ U/L	The catalytic-activity concentration of guanosine deaminase in Mr. Smith's plasma is 250 mU/L.
10^3 enzyme unit per litre	kU/L'	$\times 10^3$ U/L	The catalytic-activity concentration of pancreatic amylase in Mr. Smith's duodenal fluid is 40×10^3 U/L.

unit. 'IU' should not be confused with the symbol for enzyme unit 'U' that is defined as 'µmol per minute' (5).

A current CRM may not be permanent for a specific measurand, and the magnitude of the unit may be redefined by a new CRM batch (see examples below). To distinguish between different IUs, the given CRM should be stated in the examination report.

In the NPU terminology, the specific CRM is a part of the laboratory examination code (in the examples below 'IS 09/172' and 'IS 84/665' are specific CRMs).

Examples

NPU58076 Plasma—Coagulation factor IX; arbitrary substance concentration (enzymatic; IS 09/172; procedure) = ? IU/L

NPU01636 Plasma—Coagulation factor IX; arbitrary substance concentration (enzymatic; IS 84/665; procedure) = ? IU/L

Note: The modifier 'arbitrary' is ambiguous. Sometimes it is used for 'random'. This is not the case here. An 'arbitrary substance concentration' is a substance concentration decided and defined by an 'arbiter'. In this case 'WHO' is the 'arbiter'.

The use of SI prefix factors is allowed in descriptions of very small or very large values, because the international CRM has a well-defined magnitude. However, SI prefixes are not recommended in combination with IU expressions due to confusion with the symbol for the 'enzyme unit', U (Table 11). E.g. 'kU/L' can be mistaken for 'kIU/L', and 'mU/L' for 'mIU/L'.

6.2 Procedure defined unit (p.d.u.)

If the unit is defined by a measurement procedure that is not traceable to an international unit or an international CRM, the laboratory must describe and term the unit used. Such units are frequently termed 'arbitrary unit', 'arbitrary unit/L', 'ELISA unit', etc. — without any indication of either dimension or magnitude.

The NPU terminology uses the term '(procedure defined unit)', symbolized '(p.d.u.)', to indicate that the NPU terminology does not specify the unit for the kind-of-quantity in question. Although it may appear to be a well-defined unit, the concept contains a heterogeneous group of arbitrary and proprietary units.

It reflects the disagreement of the unit magnitudes between different assays and no common CRM.

The actual magnitude of the unit depends on the analytical measurement procedure, and it is the responsibility of the laboratory to communicate the required information for clinical evaluation of the laboratory reports.

Thus, the '(procedure defined unit)' is a simple placeholder for the units that one or more laboratories have termed and described.

Local symbols for these non-SI units should not look like SI-units, such as 'mg/L', to prevent misunderstanding of laboratory values.

Example

NPU29718 Plasma—3-hydroxy-3-methylglutaryl-coenzyme A reductase antibody (IgG); arbitrary substance concentration (procedure) = ? (procedure defined unit)

In this case, the local term for the '(procedure defined unit)' could be, e.g., 'arbitrary unit/L'.

Combinations of the term '(p.d.u.)' with SI prefixes and/or SI- or non-SI units are meaningless, as they may represent units of any magnitude and dimension (Table 12).

Comparisons on a national or regional level require harmonisation and pre-coordination for the laboratory examinations using '(p.d.u.)' as unit.

Table 12 Examples of use of procedure defined unit

Unit	Unit symbol	Examples of deprecated symbols	Examination example
Procedure defined unit	(p.d.u.)	(p.d.u.)/kg	The arbitrary substance content of haemoglobin in Mr. Smith's faeces is 20 ELISA unit/kg.
		m(p.d.u.)	
		10^{-3}× (p.d.u.)	

7. Exceptions

Units that violate some of the above rules may exceptionally be accepted as follows.

7.1 International recommendation on specific units

Well-defined and unambiguous units that violate the above stated rules may be acceptable for use if an international recommendation has been established.

Example

'Millimole per mole' ('mmol/mol') was recommended by IFCC for the laboratory examination of 'HbA_{1C}' (9).

7.2 Per cent

Many kinds-of-quantity defined as fractions are by convention and very long tradition expressed with the unit 'per cent' ('%' or '10^{-2}'), however, it is recommended to use caution when using this unit due to the high risk of errors in communication between health personnel, as explained in section 5. Therefore, if there is a strong international need of using '%' as unit for a specific laboratory examination, an international recommendation needs to be established for that specific laboratory examination.

Example

'Per cent' was recommended by IFCC for the laboratory examination 'carbohydrate-deficient transferrin (CDT)' (10).

NPU57406 Transferrin (Plasma)—Disialotransferrin; substance fraction (IFCC 2016) = ? %

Consequently, for the NPU terminology, NPU codes for that laboratory examination, using 'one' or '10^{-3}' as units, cannot be established due to risk of misinterpretation of exchanged laboratory results. This will ensure that only '%' will be reported in any laboratory.

7.3 Units lifted to a power

For units lifted to a power, e.g. 'cm^2' and 'm^3', the SI prefixes with a factor less than 1000 are acceptable for a laboratory examination with the same system, component, and kind-of-quantity. E.g. 'mm^2', 'cm^2', 'dm^2' and 'm^2' are acceptable, because they ensure steps of at least a factor of 100 between the numerical values.

The intervals of the value sets for these units are not overlapping, and there is no increased risk of misinterpretation in exchanging laboratory reports.

Examples

Patient—Body Surface; area = 1.8 m^2

Patient—Body Surface; area = 180 dm^2

Patient—Body Surface; area = 18 000 cm^2

Patient—Body Surface; area = 1 800 000 mm^2

Note: The two bottom entries should for readability purposes not be established (see Section 3: Multiples and submultiples of units).

CONCLUSION

Globally, millions of laboratory examinations are performed, communicated, exchanged, and presented every day. Moreover, as patients (and health care personnel) are traveling between hospitals and other health care organisations, patient health data are communicated between these organisations as well.

The risk of post-analytical misinterpretations – especially of the exchanged laboratory data – is, thus, high and may induce errors in patient care. To reduce risk and support optimal interoperability, the reviewed principles on measurement units are recommended for use by all parties in health care IT systems and organisations, and in scientific publications in the field of health care.

To illustrate our recommendations regarding measurement units, we provide a list of two hundred frequent laboratory examinations with units as used in Danish, Dutch, Norwegian, and Swedish laboratories. See Supplement to 'measurement units' (in Table 13, after the References section).

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In memory of Rene Dybkær and his tremendous contribution to laboratory medicine.

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Vocabulary

component: *part of a system (5)*

kind-of-nominal-property: *defining aspect, common to mutually comparable nominal properties (11)*

kind-of-quantity: *aspect common to mutually comparable quantities (6)*

nominal property: *property of a phenomenon, body, or substance, where the property has no size (11)*

numerical quantity value: *(numerical value, value): number in the expression of a quantity value, other than any number serving as the reference (6)*

ordinal kind-of-quantity: *quantity, defined by a conventional measurement procedure, for which a total ordering relation can be established, according to magnitude, with other quantities of the same kind, but for which no algebraic operations among those quantities exist (6)*

quantity value: *number and reference together expressing magnitude of a quantity (6)*

system: *part or phenomenon of the perceivable or conceivable world consisting of a demarcated arrangement of a set of elements and a set of relations or processes between these elements (5)*

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Table 13 Supplement to 'measurement units'

Rank (see commentary)	*Non-authorized indications (trivial terms and abbreviations)	**NPU identifier	Comprehensive, systematic NPU term of laboratory examinations	Abbreviated NPU term of laboratory examinations	System	Sys-spec.	Prefix	Component	Comp-spec.	Kind-of-property"	Procedure	Unit	Speciality	Scale type
1		NPU03230	Plasma—Potassium ion; substance concentration = ? mmol/L	P—Potassium ion; subst.c. = ? mmol/L	Plasma			Potassium ion		substance concentration		mmol/L	Clinical Biochemistry	Ratio
2		NPU28309	Blood—Haemoglobin; mass concentration = ? g/L	B—Haemoglobin; mass c. = ? g/L	Blood			Haemoglobin		mass concentration		g/L	Clinical Biochemistry	Ratio
3		NPU02319	Blood—Haemoglobin(Fe); substance concentration = ? mmol/L	B—Haemoglobin(Fe); subst.c. = ? mmol/L	Blood			Haemoglobin	Fe	substance concentration		mmol/L	Clinical Biochemistry	Ratio
4		NPU03429	Plasma—Sodium ion; substance concentration = ? mmol/L	P—Sodium ion; subst.c. = ? mmol/L	Plasma			Sodium ion		substance concentration		mmol/L	Clinical Biochemistry	Ratio
5		NPU02593	Blood—Leukocytes; number concentration = ? × 109/L	B—Leukocytes; num.c. = ? × 10⁹/L	Blood			Leukocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
6	ALAT	NPU19651	Plasma—Alanine transaminase; catalytic concentration(IFCC 2002) = ? U/L	P—Alanine transaminase; cat.c.(IFCC 2002) = ? U/L	Plasma			Alanine transaminase		catalytic concentration	IFCC 2002	U/L	Clinical Biochemistry	Ratio
7	CRP	NPU19748	Plasma—C-reactive protein; mass concentration = ? mg/L	P—C-reactive protein; mass c. = ? mg/L	Plasma			C-reactive protein		mass concentration		mg/L	Clinical Biochemistry	Ratio
8	Platelets	NPU03568	Blood—Thrombocytes; number concentration = ? × 109/L	B—Thrombocytes; num.c. = ? × 10⁹/L	Blood			Thrombocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
9		NPU18016	Plasma—Creatininium; substance concentration = ? µmol/L	P—Creatininium; subst.c. = ? µmol/L	Plasma			Creatininium		substance concentration		µmol/L	Clinical Biochemistry	Ratio
10	ALP	NPU27783	Plasma—Alkaline phosphatase; catalytic concentration(37 °C; procedure) = ? U/L	P—Alkaline phosphatase; cat.c.(37 °C; proc.) = ? U/L	Plasma			Alkaline phosphatase		catalytic concentration	37 °C; procedure	U/L	Clinical Biochemistry	Ratio
11		NPU19673	Plasma—Albumin; mass concentration(procedure) = ? g/L	P—Albumin; mass c.(proc.) = ? g/L	Plasma			Albumin		mass concentration	procedure	g/L	Clinical Biochemistry	Ratio
12	ALAT	NPU19981	Plasma—Alanine transaminase; catalytic concentration(IFCC 2002) = ? µkat/L	P—Alanine transaminase; cat.c.(IFCC 2002) = ? µkat/L	Plasma			Alanine transaminase		catalytic concentration	IFCC 2002	µkat/L	Clinical Biochemistry	Ratio
13	ALP	NPU01144	Plasma—Alkaline phosphatase; catalytic concentration(37 °C; procedure) = ? µkat/L	P—Alkaline phosphatase; cat.c.(37 °C; proc.) = ? µkat/L	Plasma			Alkaline phosphatase		catalytic concentration	37 °C; procedure	µkat/L	Clinical Biochemistry	Ratio
14		NPU01933	Blood—Eosinophilocytes; number concentration = ? × 109/L	B—Eosinophilocytes; num.c. = ? × 10⁹/L	Blood			Eosinophilocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
15		NPU02636	Blood—Lymphocytes; number concentration = ? × 109/L	B—Lymphocytes; num.c. = ? × 10⁹/L	Blood			Lymphocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
16		NPU02840	Blood—Monocytes; number concentration = ? × 109/L	B—Monocytes; num.c. = ? × 10⁹/L	Blood			Monocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
17		NPU01349	Blood—Basophilocytes; number concentration = ? × 109/L	B—Basophilocytes; num.c. = ? × 10⁹/L	Blood			Basophilocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
18		NPU04998	Plasma—Creatininium; substance concentration(enzymatic) = ? µmol/L	P—Creatininium; subst.c.(enz.) = ? µmol/L	Plasma			Creatininium		substance concentration	enzymatic	µmol/L	Clinical Biochemistry	Ratio

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19	ASAT	NPU22279	Plasma—Aspartate transaminase; catalytic concentration(IFCC 2002) = ? µkat/L	P—Aspartate transaminase; cat.c.(IFCC 2002) = ? µkat/L	Plasma			Aspartate transaminase		catalytic concentration	IFCC 2002	µkat/L	Clinical Biochemistry	Ratio
20		NPU01370	Plasma—Bilirubins; substance concentration = ? µmol/L	P—Bilirubins; subst.c. = ? µmol/L	Plasma			Bilirubins		substance concentration		µmol/L	Clinical Biochemistry	Ratio
21		NPU02902	Blood—Neutrophilocytes; number concentration = ? × 109/L	B—Neutrophilocytes; num.c. = ? × 10⁹/L	Blood			Neutrophilocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
22	HbA1c (IFCC)	NPU27300	Haemoglobin beta chain(Blood)—N-(1-deoxyfructos-1-yl)haemoglobin beta chain; substance fraction = ? mmol/mol	Haemoglobin beta chain(B)—N-(1-deoxyfructos-1-yl)haemoglobin beta chain; subst.fr. = ? mmol/mol	Haemoglobin beta chain	Blood		N-(1-deoxyfructos-1-yl) haemoglobin beta chain		substance fraction		mmol/mol	Clinical Biochemistry	Ratio
23	eAG (estimated Average Glucose)	NPU27412	Plasma—Glucose; substance concentration(average; Hb A1c; procedure) = ? mmol/L	P—Glucose; subst.c.(average; Hb A1c; proc.) = ? mmol/L	Plasma			Glucose		substance concentration	average; Hb A1c; procedure	mmol/L	Clinical Biochemistry	Ratio
24		NPU01459	Plasma—Carbamide; substance concentration = ? mmol/L	P—Carbamide; subst.c. = ? mmol/L	Plasma			Carbamide		substance concentration		mmol/L	Clinical Biochemistry	Ratio
25	TSH	NPU03577	Plasma—Thyrotropin; arbitrary substance concentration(IRP 80/558; procedure) = ? × 10-3 IU/L	P—Thyrotropin; arb.subst.c.(IRP 80/558; proc.) = ? × 10⁻³ IU/L	Plasma			Thyrotropin		arbitrary substance concentration	IRP 80/558; procedure	× 10-3 IU/L	Clinical Biochemistry	Ratio
26	HbA1c (DCCT)	NPU29296	Haemoglobin(Fe;Blood)—Haemoglobin A1c(Fe); substance fraction(NGSP) = ? %	Hb(Fe; B)—Haemoglobin A1c(Fe); subst.fr.(NGSP) = ? %	Haemoglobin	Fe; Blood		Haemoglobin A1c	Fe	substance fraction	NGSP	%	Clinical Biochemistry	Ratio
27	Total cholesterol	NPU01566	Plasma—Cholesterol+ester; substance concentration = ? mmol/L	P—Cholesterol+ester; subst.c. = ? mmol/L	Plasma			Cholesterol +ester		substance concentration		mmol/L	Clinical Biochemistry	Ratio
28	LDL	NPU01568	Plasma—Cholesterol+ester, in LDL; substance concentration = ? mmol/L	P—Cholesterol+ester, in LDL; subst.c. = ? mmol/L	Plasma			Cholesterol +ester, in LDL		substance concentration		mmol/L	Clinical Biochemistry	Ratio
29	HDL	NPU01567	Plasma—Cholesterol+ester, in HDL; substance concentration = ? mmol/L	P—Cholesterol+ester, in HDL; subst.c. = ? mmol/L	Plasma			Cholesterol +ester, in HDL		substance concentration		mmol/L	Clinical Biochemistry	Ratio
30	GGT	NPU22283	Plasma—gamma-Glutamyltransferase; catalytic concentration(IFCC 2002) = ? µkat/L	P—gamma-Glutamyltransferase; cat.c.(IFCC 2002) = ? µkat/L	Plasma		gamma-	Glutamyl-transferase		catalytic concentration	IFCC 2002	µkat/L	Clinical Biochemistry	Ratio
31		NPU26880	Erythrocytes(Blood)—Haemoglobin; entitic mass = ? pg	Ercs(B)—Haemoglobin; entitic mass = ? pg	Erythrocytes	Blood		Haemoglobin		entitic mass		pg	Clinical Biochemistry	Ratio
32		NPU26631	Blood—Metamyelocytes+Myelocytes +Promyelocytes; number concentration = ? × 109/L	B—Metamyelocytes+Myelocytes+Promyelocytes; num.c. = ? × 10⁹/L	Blood			Metamyelocytes + Myelocytes + Promyelocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio

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33	LDH	NPU19658	Plasma—L-Lactate dehydrogenase; catalytic concentration(IFCC 2002) = ? U/L	P—L-Lactate dehydrogenase; cat.c.(IFCC 2002) = ? U/L	Plasma		L-	Lactate dehydrogenase		catalytic concentration	IFCC 2002	U/L	Clinical Biochemistry	Ratio
34	Triglycerides	NPU04094	Plasma—Triglyceride; substance concentration = ? mmol/L	P—Triglyceride; subst.c. = ? mmol/L	Plasma			Triglyceride		substance concentration		mmol/L	Clinical Biochemistry	Ratio
35	MCV	NPU01944	Blood—Erythrocytes; entitic volume = ? fL	B—Erythrocytes; entitic vol. = ? fL	Blood			Erythrocytes		entitic volume		fL	Clinical Biochemistry	Ratio
36	Haematocrit	NPU01961	Blood—Erythrocytes; volume fraction = ?	B—Erythrocytes; vol.fr. = ?	Blood			Erythrocytes		volume fraction			Clinical Biochemistry	Ratio
37	Calcium	NPU01443	Plasma—Calcium(II); substance concentration = ? mmol/L	P—Calcium(II); subst.c. = ? mmol/L	Plasma			Calcium	II	substance concentration		mmol/L	Clinical Biochemistry	Ratio
38	Vitamin B12	NPU01700	Plasma—Cobalamin; substance concentration = ? pmol/L	P—Cobalamin; subst.c. = ? pmol/L	Plasma			Cobalamin		substance concentration		pmol/L	Clinical Biochemistry	Ratio
39	Calcium ion	NPU04144	Plasma—Calcium ion(free); substance concentration(pH = 7.40;procedure) = ? mmol/L	P—Calcium ion(free); subst.c.(pH = 7.40; proc.) = ? mmol/L	Plasma			Calcium ion	free	substance concentration	pH = 7.40; procedure	mmol/L	Clinical Biochemistry	Ratio
40		NPU02192	Plasma—Glucose; substance concentration = ? mmol/L	P—Glucose; subst.c. = ? mmol/L	Plasma			Glucose		substance concentration		mmol/L	Clinical Biochemistry	Ratio
41	MCHC	NPU02321	Erythrocytes(Blood)—Haemoglobin(Fe); substance concentration = ? mmol/L	Ercs(B)—Haemoglobin(Fe); subst.c. = ? mmol/L	Erythrocytes	Blood		Haemoglobin	Fe	substance concentration		mmol/L	Clinical Biochemistry	Ratio
42	GGT	NPU19657	Plasma—gamma-Glutamyltransferase; catalytic concentration(IFCC 2002) = ? U/L	P—gamma-Glutamyltransferase; cat.c.(IFCC 2002) = ? U/L	Plasma		gamma-	Glutamyltransferase		catalytic concentration	IFCC 2002	U/L	Clinical Biochemistry	Ratio
43	Prothrombine time	NPU18878	Plasma—Coagulation, tissue factor-induced; arbitrary substance concentration(coagulation; procedure) = ? (p.d.u.)	P—Coagulation, tissue factor-induced; arb. subst.c.(coag.; proc.) = ? (p.d.u.)	Plasma			Coagulation, tissue factor-induced		arbitrary substance concentration	coagulation; procedure	(p.d.u.)	Trombosis and Haemostasis	Ratio
44	Vitamin D2+D3	NPU10267	Plasma—Calcifediol+25-Hydroxyergocalciferol; substance concentration = ? nmol/L	P—Calcifediol+25-Hydroxyergocalciferol; subst.c. = ? nmol/L	Plasma			Calcifediol+25-Hydroxyergocalciferol		substance concentration		nmol/L	Clinical Biochemistry	Ratio
45		NPU01960	Blood—Erythrocytes; number concentration = ? × 1012/L	B—Erythrocytes; num.c. = ? × 10¹²/L	Blood			Erythrocytes		number concentration		× 1012/L	Clinical Biochemistry	Ratio
46	25-Hydroxy-Vitamin D2	NPU26810	Plasma—25-Hydroxyergocalciferol; substance concentration = ? nmol/L	P—25-Hydroxyergocalciferol; subst.c. = ? nmol/L	Plasma		25-	Hydroxyergocalciferol		substance concentration		nmol/L	Clinical Biochemistry	Ratio
47		NPU19763	Plasma—Ferritin; mass concentration = ? µg/L	P—Ferritin; mass c. = ? µg/L	Plasma			Ferritin		mass concentration		µg/L	Clinical Biochemistry	Ratio
48		NPU19653	Plasma—Amylase, pancreatic type; catalytic concentration(IFCC 2006) = ? U/L	P—Amylase, pancreatic type; cat.c.(IFCC 2006) = ? U/L	Plasma			Amylase, pancreatic type		catalytic concentration	IFCC 2006	U/L	Clinical Biochemistry	Ratio
49		NPU02508	Plasma—Iron; substance concentration = ? µmol/L	P—Iron; subst.c. = ? µmol/L	Plasma			Iron		substance concentration		µmol/L	Clinical Biochemistry	Ratio

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50		NPU03096	Plasma—Phosphate(P; inorganic); substance concentration = ? mmol/L	P—Phosphate(P; inorganic); subst.c. = ? mmol/L	Plasma			Phosphate (P; inorganic)		substance concentration		mmol/L	Clinical Biochemistry	Ratio
51		NPU03688	Plasma—Urate; substance concentration = ? mmol/L	P—Urate; subst.c. = ? mmol/L	Plasma			Urate		substance concentration		mmol/L	Clinical Biochemistry	Ratio
52		NPU04133	Plasma—Iron binding capacity; substance concentration = ? µmol/L	P—Iron binding capacity; subst.c. = ? µmol/L	Plasma			Iron binding capacity		substance concentration		µmol/L	Clinical Biochemistry	Ratio
53		NPU19652	Plasma—Amylase; catalytic concentration(IFCC 2006) = ? U/L	P—Amylase; cat.c.(IFCC 2006) = ? U/L	Plasma			Amylase		catalytic concentration	IFCC 2006	U/L	Clinical Biochemistry	Ratio
54	Free T4	NPU03579	Plasma—Thyroxine(free); substance concentration = ? pmol/L	P—Thyroxine(free); subst.c. = ? pmol/L	Plasma			Thyroxine	free	substance concentration		pmol/L	Clinical Biochemistry	Ratio
55	LDH	NPU22289	Plasma—L-Lactate dehydrogenase; catalytic concentration(IFCC 2002) = ? µkat/L	P—L-Lactate dehydrogenase; cat.c.(IFCC 2002) = ? µkat/L	Plasma		L-	Lactate dehydrogenase		catalytic concentration	IFCC 2002	µkat/L	Clinical Biochemistry	Ratio
56	Urinary albumin excretion adjusted for creatinine	NPU19661	Urine—Albumin/Creatininium; mass ratio = ? × 10-3 IU/L	U—Albumin/Creatininium; mass ratio = ? × 10⁻³	Urine			Albumin/ Creatininium		mass ratio		× 10-3 IU/L	Clinical Biochemistry	Ratio
57		NPU19986	Plasma—Amylase, pancreatic type; catalytic concentration(IFCC 2006) = ? µkat/L	P—Amylase, pancreatic type; cat.c.(IFCC 2006) = ? µkat/L	Plasma			Amylase, pancreatic type		catalytic concentration	IFCC 2006	µkat/L	Clinical Biochemistry	Ratio
58	MCH	NPU02320	Erythrocytes(Blood)—Haemoglobin(Fe); entitic amount-of-substance = ? fmol	Ercs(B)—Haemoglobin(Fe); entitic am.s. = ? fmol	Erythro-cytes	Blood		Haemoglobin	Fe	entitic amount-of-substance		fmol	Clinical Biochemistry	Ratio
59		NPU08694	Blood—Reticulocytes; number concentration = ? × 109/L	B—Reticulocytes; num.c. = ? × 10⁹/L	Blood			Reticulocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
60	Adjusted Calcium	NPU04169	Plasma—Calcium(II); substance concentration (adjusted; procedure) = ? mmol/L	P—Calcium(II); subst.c.(adj.; proc.) = ? mmol/L	Plasma			Calcium	II	substance concentration	adjusted; procedure	mmol/L	Clinical Biochemistry	Ratio
61		NPU02070	Plasma—Folate; substance concentration = ? nmol/L	P—Folate; subst.c. = ? nmol/L	Plasma			Folate		substance concentration		nmol/L	Clinical Biochemistry	Ratio
62		NPU04073	Plasma—Homocysteine; substance concentration = ? µmol/L	P—Homocysteine; subst.c. = ? µmol/L	Plasma			Homocysteine		substance concentration		µmol/L	Clinical Biochemistry	Ratio
63		NPU22089	Plasma(cord Blood)—Glucose; substance concentration = ? mmol/L	P(cB)—Glucose; subst.c. = ? mmol/L	Plasma	cord Blood		Glucose		substance concentration		mmol/L	Clinical Biochemistry	Ratio
64		NPU02647	Plasma—Magnesium(II); substance concentration = ? mmol/L	P—Magnesium(II); subst.c. = ? mmol/L	Plasma			Magnesium	II	substance concentration		mmol/L	Clinical Biochemistry	Ratio
65	Pro-BNP	NPU21571	Plasma—Pro-brain natriuretic peptide(1-76); mass concentration = ? ng/L	P—Pro-brain natriuretic peptide(1-76); mass c. = ? ng/L	Plasma			Pro-brain natriuretic peptide(1-76)		mass concentration		ng/L	Clinical Biochemistry	Ratio
66	pCO2	NPU01470	Plasma(Arterial blood)—Carbon dioxide; tension(37 °C) = ? kPa	P(aB)—Carbon dioxide; tension(37 °C) = ? kPa	Plasma	Arterial blood		Carbon dioxide		tension	37 °C	kPa	Clinical Biochemistry	Ratio

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67		NPU09105	Plasma—Calcifediol+ergocalciferol; substance concentration = ? nmol/L	P—Calcifediol+ergocalciferol; subst.c. = ? nmol/L	Plasma			Calcifediol +ergocalciferol		substance concentration		nmol/L	Clinical Biochemistry	Ratio
68	pO2	NPU08977	Plasma(Arterial blood)—Oxygen(O₂); tension (37 °C) = ? kPa	P(aB)—Oxygen(O₂); tension(37 °C) = ? kPa	Plasma	Arterial blood		Oxygen	O₂	tension	37 °C	kPa	Clinical Biochemistry	Ratio
69	CK	NPU19656	Plasma—Creatine kinase; catalytic concentration(IFCC 2002) = ? U/L	P—Creatine kinase; cat.c.(IFCC 2002) = ? U/L	Plasma			Creatine kinase		catalytic concentration	IFCC 2002	U/L	Clinical Biochemistry	Ratio
70		NPU09102	Urine—Creatininium; substance concentration = ? mmol/L	U—Creatininium; subst.c. = ? mmol/L	Urine			Creatininium		substance concentration		mmol/L	Clinical Biochemistry	Ratio
71		NPU28172	Blood—Neutrophilocytes(segmented+band); number concentration = ? × 109/L	B—Neutrophilocytes(segmented+band); num.c. = ? × 10⁹/L	Blood			Neutrophilocytes	seg-mented + band	number concentration		× 109/L	Clinical Biochemistry	Ratio
72		NPU03943	Plasma(Arterial blood)—Lactate; substance concentration = ? mmol/L	P(aB)—Lactate; subst.c. = ? mmol/L	Plasma	Arterial blood		Lactate		substance concentration		mmol/L	Clinical Biochemistry	Ratio
73		NPU19677	Urine—Albumin; mass concentration(procedure) = ? mg/L	U—Albumin; mass c.(proc.) = ? mg/L	Urine			Albumin		mass concentration	procedure	mg/L	Clinical Biochemistry	Ratio
74		NPU28842	Urine—Albumin/Creatininium; mass coefficient(mass/amount-of-substance;procedure) = ? g/mol	U—Albumin/Creatininium; mass coefficient(mass/am.s.; proc.) = ? g/mol	Urine			Albumin/ Creatininium		mass coefficient	mass/amount-of-substance; procedure sprocedure-substance; procedure substance; esubstance; procedure	g/mol	Clinical Biochemistry	Ratio
75	VLDL	NPU01569	Plasma—Cholesterol+ester, in VLDL; substance concentration = ? mmol/L	P—Cholesterol+ester, in VLDL; subst.c. = ? mmol/L	Plasma			Cholesterol +ester, in VLDL		substance concentration		mmol/L	Clinical Biochemistry	Ratio
76		NPU04191	Transferrin(Fe-binding sites;Plasma)—Iron; substance fraction = ?	Transferrin(Fe-binding sites; P)—Iron; subst.fr. = ?	Transfer-rin	Fe-binding sites; Plasma		Iron		substance fraction			Clinical Biochemistry	Ratio
77	CO2	NPU01472	Plasma(Venous blood)—Carbon dioxide; substance concentration = ? mmol/L	P(vB)—Carbon dioxide; subst.c. = ? mmol/L	Plasma	Venous blood		Carbon dioxide		substance concentration		mmol/L	Clinical Biochemistry	Ratio
78	Urine pH	NPU02415	Urine—Hydrogen ion; pH(procedure) = ?	U—Hydrogen ion; pH(proc.) = ?	Urine			Hydrogen ion		pH	procedure		Clinical Biochemistry	Loga-rith-mic
79	Fasting triglycerides	NPU03620	Plasma(fasting Patient)—Triglyceride; substance concentration = ? mmol/L	P(fPt)—Triglyceride; subst.c. = ? mmol/L	Plasma	fasting Patient		Triglyceride		substance concentration		mmol/L	Clinical Biochemistry	Ratio
80	Base excess	NPU03815	Extracellular fluid—Base excess; substance concentration(actual-norm) = ? mmol/L	Ecf—Base excess; subst.c.(actual-norm) = ? mmol/L	Extracel-lular fluid			Base excess		substance concentration	actual-norm	mmol/L	Clinical Biochemistry	Dif-feren-tial
81	HbA1c	NPU03835	Haemoglobin(Fe;Blood)—Haemoglo-bin A1c(Fe); substance fraction = ?	Hb(Fe; B)—Haemoglobin A1c(Fe); subst.fr. = ?	Haemo-globin	Fe; Blood		Haemoglobin A1c	Fe	substance fraction			Clinical Biochemistry	Ratio

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82	Free T3	NPU03625	Plasma—Triiodothyronine(free); substance concentration = ? pmol/L	P—Triiodothyronine(free); subst.c. = ? pmol/L	Plasma			Triiodothyronine	free	substance concentration		pmol/L	Clinical Biochemistry	Ratio
83	T3	NPU03624	Plasma—Triiodothyronine; substance concentration = ? nmol/L	P—Triiodothyronine; subst.c. = ? nmol/L	Plasma			Triiodothyronine		substance concentration		nmol/L	Clinical Biochemistry	Ratio
84	T4	NPU03578	Plasma—Thyroxine; substance concentration = ? nmol/L	P—Thyroxine; subst.c. = ? nmol/L	Plasma			Thyroxine		substance concentration		nmol/L	Clinical Biochemistry	Ratio
85	TPO antibodies	NPU20041	Plasma—Thyroid peroxidase antibody; arbitrary substance concentration(IRP 66/387; procedure) = ? × 10^3 IU/L	P—Thyroid peroxidase antibody; arb.subst.c.(IRP 66/387; proc.) = ? × 10^3 IU/L	Plasma			Thyroid peroxidase antibody		arbitrary substance concentration	IRP 66/387; procedure	× 10^3 IU/L	Clinical Biochemistry	Ratio
86	Hb in Faeces	NPU29057	Faeces—Haemoglobin; arbitrary substance concentration (procedure) = ? (p.d.u.)	F—Haemoglobin; arb.subst.c.(proc.) = ? (p.d.u.)	Faeces			Haemoglobin		arbitrary substance concentration	procedure	(p.d.u.)	Clinical Biochemistry	Ratio
87	PSA	NPU08669	Plasma—Prostata specific antigen; mass concentration = ? µg/L	P—Prostata specific antigen; mass c. = ? µg/L	Plasma			Prostata specific antigen		mass concentration		µg/L	Clinical Biochemistry	Ratio
88	activated partial thromboplastin time (APTT)	NPU01682	Plasma—Coagulation, surface-induced; time(procedure) = ? s	P—Coagulation, surface-induced; time(proc.) = ? s	Plasma			Coagulation, surface-induced		time	procedure	s	Trombosis and Haemostasis	Ratio
89	RDW-CV	NPU18162	Erythrocytes(Blood)—Erythrocyte volumes; relative distribution width(procedure) = ?	Ercs(B)—Erythrocyte volumes; relative distribution width(proc.) = ?	Erythrocytes	Blood		Erythrocyte volumes		relative distribution width	procedure		Clinical Biochemistry	Ratio
90		NPU14267	Blood—Large unstained cells; number concentration = ? × 109/L	B—Large unstained cells; num.c. = ? × 10⁹/L	Blood			Large unstained cells		number concentration		× 109/L	Clinical Biochemistry	Ratio
91	PTH	NPU03028	Plasma—Parathyrin; substance concentration = ? pmol/L	P—Parathyrin; subst.c. = ? pmol/L	Plasma			Parathyrin		substance concentration		pmol/L	Clinical Biochemistry	Ratio
92	ASAT	NPU19654	Plasma—Aspartate transaminase; catalytic concentration(IFCC 2002) = ? U/L	P—Aspartate transaminase; cat.c.(IFCC 2002) = ? U/L	Plasma			Aspartate transaminase		catalytic concentration	IFCC 2002	U/L	Clinical Biochemistry	Ratio
93	IgE	NPU56406	Plasma—Immunoglobulin E; arbitrary substance concentration(IS 11/234 ;procedure) = ? × 10^3 IU/L	P—Immunoglobulin E; arb.subst.c.(IS 11/234; proc.) = ? × 10^3 IU/L	Plasma			Immunoglobulin E		arbitrary substance concentration	IS 11/234; procedure	× 10^3 IU/L	Clinical Allergology	Ratio
94		NPU26470	Plasma—Transferrin; mass concentration = ? g/L	P—Transferrin; mass c. = ? g/L	Plasma			Transferrin		mass concentration		g/L	Clinical Biochemistry	Ratio
95		NPU21533	Plasma(Arterial blood)—Glucose; substance concentration = ? mmol/L	P(aB)—Glucose; subst.c. = ? mmol/L	Plasma	Arterial blood		Glucose		substance concentration		mmol/L	Clinical Biochemistry	Ratio
96		NPU18410	Plasma—Cholesterol+ester/ Cholesterol+ester, in HDL; substance ratio = ?	P—Cholesterol+ester/ Cholesterol+ester, in HDL; subst.ratio = ?	Plasma			Cholesterol + ester / Cholesterol + ester, in HDL		substance ratio			Clinical Biochemistry	Ratio

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97	IgG	NPU19814	Plasma—Immunoglobulin G; mass concentration = ? g/L	P—Immunoglobulin G; mass c. = ? g/L	Plasma			Immunoglobulin G		mass concentration		g/L	Clinical Biochemistry	Ratio
98		NPU10762	Blood—Atypical cells; number concentration = ? × 109/L	B—Atypical cells; num.c. = ? × 10⁹/L	Blood			Atypical cells		number concentration		× 109/L	Clinical Biochemistry	Ratio
99	IgA	NPU19795	Plasma—Immunoglobulin A; mass concentration = ? g/L	P—Immunoglobulin A; mass c. = ? g/L	Plasma			Immunoglobulin A		mass concentration		g/L	Clinical Biochemistry	Ratio
100		NPU03607	Plasma—Transferrin; substance concentration = ? µmol/L	P—Transferrin; subst.c. = ? µmol/L	Plasma			Transferrin		substance concentration		µmol/L	Clinical Biochemistry	Ratio
101	TSH	NPU27547	Plasma—Thyrotropin; arbitrary substance concentration(IRP 81/565;procedure) = ? × 10-3 IU/L	P—Thyrotropin; arb.subst.c.(IRP 81/565; proc.) = ? × 10⁻³ IU/L	Plasma			Thyrotropin		arbitrary substance concentration	IRP 81/565; procedure	× 10-3 IU/L	Clinical Biochemistry	Ratio
102	IgM	NPU19825	Plasma—Immunoglobulin M; mass concentration = ? g/L	P—Immunoglobulin M; mass c. = ? g/L	Plasma			Immunoglobulin M		mass concentration		g/L	Clinical Biochemistry	Ratio
103	HCO3	NPU02410	Plasma—Hydrogen carbonate; substance concentration (pCO₂ = 5.3 kPa; 37 °C) = ? mmol/L	P—Hydrogen carbonate; subst.c.(pCO₂ = 5.3 kPa; 37 °C) = ? mmol/L	Plasma			Hydrogen carbonate		substance concentration	pCO₂ = 5.3 kPa; 37 °C	mmol/L	Clinical Biochemistry	Ratio
104		NPU01368	Plasma—Bilirubin glucuronide; substance concentration = ? µmol/L	P—Bilirubin glucuronide; subst.c. = ? µmol/L	Plasma			Bilirubin glucuronide		substance concentration		µmol/L	Clinical Biochemistry	Ratio
105		NPU09356	Plasma—Urate; substance concentration = ? µmol/L	P—Urate; subst.c. = ? µmol/L	Plasma			Urate		substance concentration		µmol/L	Clinical Biochemistry	Ratio
106	25-Hydroxy-Vitamin D3	NPU01435	Plasma—Calcifediol; substance concentration = ? nmol/L	P—Calcifediol; subst.c. = ? nmol/L	Plasma			Calcifediol		substance concentration		nmol/L	Clinical Biochemistry	Ratio
107	O2	NPU10167	Patient—Oxygen(administered); volume rate = ? L/min	Pt—Oxygen(administered); vol.rate = ? L/min	Patient			Oxygen	administered	volume rate		L/min	Clinical Biochemistry	Ratio
108	Base excess	NPU12518	Plasma(Arterial blood)—Base excess; substance concentration(actual-norm) = ? mmol/L	P(aB)—Base excess; subst.c.(actual-norm) = ? mmol/L	Plasma	Arterial blood		Base excess		substance concentration	actual-norm	mmol/L	Clinical Biochemistry	Differential
109	A1AT	NPU19692	Plasma—alpha 1-Antitrypsin; mass concentration = ? g/L	P—alpha 1-Antitrypsin; mass c. = ? g/L	Plasma		alpha 1-	Antitrypsin		mass concentration		g/L	Clinical Biochemistry	Ratio
110	D-Dimer	NPU28289	Plasma—Fibrin D-dimer; arbitrary substance concentration(procedure) = ? (p.d.u.)	P—Fibrin D-dimer; arb.subst.c.(proc.) = ? (p.d.u.)	Plasma			Fibrin D-dimer		arbitrary substance concentration	procedure	(p.d.u.)	Trombosis and Haemostasis	Ratio
111		NPU01536	Plasma—Chloride; substance concentration = ? mmol/L	P—Chloride; subst.c. = ? mmol/L	Plasma			Chloride		substance concentration		mmol/L	Clinical Biochemistry	Ratio
112	TfR	NPU28336	Plasma—Transferrinreceptor fragment; mass concentration = ? mg/L	P—Transferrinreceptor fragment; mass c. = ? mg/L	Plasma			Transferrinreceptor fragment		mass concentration		mg/L	Clinical Biochemistry	Ratio
113	ESR	NPU03404	Blood—Sedimentation reaction; length(procedure) = ? mm	B—Sedimentation reaction; length(proc.) = ? mm	Blood			Sedimentation reaction		length	procedure	mm	Clinical Biochemistry	Ratio
114		NPU01943	Blood—Erythroblasts; number concentration = ? × 109/L	B—Erythroblasts; num.c. = ? × 10⁹/L	Blood			Erythroblasts		number concentration		× 109/L	Clinical Biochemistry	Ratio

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115		NPU23296	Urine—Buprenorphine; mass concentration = ? µg/L	U—Buprenorphine; mass c. = ? µg/L	Urine			Buprenorphine		mass concentration		µg/L	Clinical Pharmacology	Ratio
116	TNI	NPU27591	Plasma—Troponin I, cardiac muscle; mass concentration = ? ng/L	P—Troponin I, cardiac muscle; mass c. = ? ng/L	Plasma			Troponin I, cardiac muscle		mass concentration		ng/L	Clinical Biochemistry	Ratio
117		NPU01808	Urine—Creatininium; substance concentration = ? µmol/L	U—Creatininium; subst.c. = ? µmol/L	Urine			Creatininium		substance concentration		µmol/L	Clinical Biochemistry	Ratio
118	Anion gap	NPU20189	Plasma—Anion gap(excl. K+); substance concentration = ? mmol/L	P—Anion gap(excl. K+); subst.c. = ? mmol/L	Plasma			Anion gap(excl. K+)		substance concentration		mmol/L	Clinical Biochemistry	Differential
119	6-MAM	NPU24861	Urine—6-O-Monoacetylmorphine; mass concentration = ? µg/L	U—6-O-Monoacetylmorphine; mass c. = ? µg/L	Urine		6-O-	Monoacetylmorphine		mass concentration		µg/L	Clinical Pharmacology	Ratio
120		NPU03976	Blood—Myelocytes; number concentration = ? × 109/L	B—Myelocytes; num.c. = ? × 10⁹/L	Blood			Myelocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
121	CK-MB	NPU19750	Plasma—Creatine kinase MB; mass concentration = ? µg/L	P—Creatine kinase MB; mass c. = ? µg/L	Plasma			Creatine kinase MB		mass concentration		µg/L	Clinical Biochemistry	Ratio
122		NPU57688	Plasma—Food allergen antibody(IgE); arbitrary substance concentration((f1; f2; f3; f4; f13; f14);procedure) = ? (p.d.u.)	P—Food allergen antibody(IgE); arb. subst.c.((f1; f2; f3; f4; f13; f14); proc.) = ? (p.d.u.)	Plasma			Food allergen antibody	IgE	arbitrary substance concentration	(f1; f2; f3; f4; f13; f14); procedure	(p.d.u.)	Clinical Allergology	Ratio
123	THC-COOH	NPU28551	Urine—11-Nor-delta(9)-tetrahydrocannabinol-9-carboxylic acid; mass concentration = ? µg/L	U—11-Nor-delta(9)-tetrahydrocannabinol-9-carboxylic acid; mass c. = ? µg/L	Urine		11-	Nor-delta(9)-tetrahydrocannabinol-9-carboxylic acid		mass concentration		µg/L	Clinical Pharmacology	Ratio
124		NPU03978	Blood—Metamyelocytes; number concentration = ? × 109/L	B—Metamyelocytes; num.c. = ? × 10⁹/L	Blood			Metamyelocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
125		NPU19788	Plasma—Haptoglobin; mass concentration = ? g/L	P—Haptoglobin; mass c. = ? g/L	Plasma			Haptoglobin		mass concentration		g/L	Clinical Biochemistry	Ratio
126		NPU23111	Urine—Amfetamine; mass concentration = ? µg/L	U—Amfetamine; mass c. = ? µg/L	Urine			Amfetamine		mass concentration		µg/L	Clinical Pharmacology	Ratio
127	TNT	NPU27501	Plasma—Troponin T, cardiac muscle; mass concentration = ? ng/L	P—Troponin T, cardiac muscle; mass c. = ? ng/L	Plasma			Troponin T, cardiac muscle		mass concentration		ng/L	Clinical Biochemistry	Ratio
128		NPU28062	Urine—Oxazepam; mass concentration = ? µg/L	U—Oxazepam; mass c. = ? µg/L	Urine			Oxazepam		mass concentration		µg/L	Clinical Pharmacology	Ratio
129	Free PSA	NPU12534	Plasma—Prostata specific antigen(free); mass concentration = ? µg/L	P—Prostata specific antigen(free); mass c. = ? µg/L	Plasma			Prostata specific antigen	free	mass concentration		µg/L	Clinical Biochemistry	Ratio
130		NPU28061	Urine—Nordazepam; mass concentration = ? µg/L	U—Nordazepam; mass c. = ? µg/L	Urine			Nordazepam		mass concentration		µg/L	Clinical Pharmacology	Ratio

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131		NPU03972	Blood—Blast cells(unspecified); number concentration(procedure) = ? × 109/L	B—Blast cells(unspecified); num.c.(proc.) = ? × 10⁹/L	Blood			Blast cells	unspecified	number concentration	procedure	× 109/L	Clinical Biochemistry	Ratio
132		NPU28056	Urine—7-Aminoclonazepam; mass concentration = ? µg/L	U—7-Aminoclonazepam; mass c. = ? µg/L	Urine		7-	Aminoclonazepam		mass concentration		µg/L	Clinical Pharmacology	Ratio
133		NPU03974	Blood—Promyelocytes; number concentration = ? × 109/L	B—Promyelocytes; num.c. = ? × 10⁹/L	Blood			Promyelocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
134		NPU03768	Plasma—Zinc; substance concentration = ? µmol/L	P—Zinc; subst.c. = ? µmol/L	Plasma			Zinc		substance concentration		µmol/L	Clinical Biochemistry	Ratio
135		NPU28054	Urine—alpha-Hydroxyalprazolam; mass concentration = ? µg/L	U—alpha-Hydroxyalprazolam; mass c. = ? µg/L	Urine		alpha-	Hydroxyalprazolam		mass concentration		µg/L	Clinical Pharmacology	Ratio
136	hCG+beta chain	NPU19579	Plasma—Choriogonadotropin+beta-chain; arbitrary substance concentration(IS 75/589;procedure) = ? IU/L	P—Choriogonadotropin+beta-chain; arb.subst.c.(IS 75/589; proc.) = ? IU/L	Plasma			Choriogonadotropin +beta-chain		arbitrary substance concentration	IS 75/589; procedure	IU/L	Clinical Biochemistry	Ratio
137		NPU28057	Urine—7-Aminonitrazepam; mass concentration = ? µg/L	U—7-Aminonitrazepam; mass c. = ? µg/L	Urine		7-	Aminonitrazepam		mass concentration		µg/L	Clinical Pharmacology	Ratio
138		NPU19676	Urine—Albumin; mass concentration(procedure) = ? g/L	U—Albumin; mass c.(proc.) = ? g/L	Urine			Albumin		mass concentration	procedure	g/L	Clinical Biochemistry	Ratio
139		NPU24776	Urine—Metamfetamine; mass concentration = ? µg/L	U—Metamfetamine; mass c. = ? µg/L	Urine			Metamfetamine		mass concentration		µg/L	Clinical Pharmacology	Ratio
140		NPU03278	Plasma—Protein; mass concentration = ? g/L	P—Protein; mass c. = ? g/L	Plasma			Protein		mass concentration		g/L	Clinical Biochemistry	Ratio
141		NPU28055	Urine—7-Aminoflunitrazepam; mass concentration = ? µg/L	U—7-Aminoflunitrazepam; mass c. = ? µg/L	Urine		7-	Aminoflunitrazepam		mass concentration		µg/L	Clinical Pharmacology	Ratio
142	Anion gap	NPU18415	Plasma—Anion gap(incl. K+); substance concentration = ? mmol/L	P—Anion gap(incl. K+); subst.c. = ? mmol/L	Plasma			Anion gap(incl. K+)		substance concentration		mmol/L	Clinical Biochemistry	Differential
143		NPU54550	Urine—Ephedrine; mass concentration = ? µg/L	U—Ephedrine; mass c. = ? µg/L	Urine			Ephedrine		mass concentration		µg/L	Clinical Pharmacology	Ratio
144		NPU03356	Erythrocytes(Blood)—Reticulocytes; number fraction = ? × 10-3 IU/L	Ercs(B)—Reticulocytes; num.fr. = ? × 10⁻³	Erythrocytes	Blood		Reticulocytes		number fraction		× 10-3 IU/L	Clinical Biochemistry	Ratio
145		NPU54587	Urine—4-Methoxyamphetamine; mass concentration = ? µg/L	U—4-Methoxyamphetamine; mass c. = ? µg/L	Urine		4-	Methoxyamphetamine		mass concentration		µg/L	Clinical Pharmacology	Ratio
146	FSH	NPU04014	Plasma—Follitropin; arbitrary substance concentration(IRP 78/549; procedure) = ? IU/L	P—Follitropin; arb.subst.c.(IRP 78/549; proc.) = ? IU/L	Plasma			Follitropin		arbitrary substance concentration	IRP 78/549; procedure	IU/L	Clinical Biochemistry	Ratio
147		NPU54749	Urine—4-Methoxymethamphetamine; mass concentration = ? µg/L	U—4-Methoxymethamphetamine; mass c. = ? µg/L	Urine		4-	Methoxymethamphetamine		mass concentration		µg/L	Clinical Pharmacology	Ratio

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148	HCO3	NPU14266	Plasma(Venous blood)—Hydrogen carbonate; substance concentration(actual;37 °C) = ? mmol/L	P(vB)—Hydrogen carbonate; subst.c.(actual; 37 °C) = ? mmol/L	Plasma	Venous blood		Hydrogen carbonate		substance concentration	actual; 37 °C	mmol/L	Clinical Biochemistry	Ratio
149		NPU28311	Urine—Benzoylecgonine; mass concentration = ? µg/L	U—Benzoylecgonine; mass c. = ? µg/L	Urine			Benzoylecgonine		mass concentration		µg/L	Clinical Pharmacology	Ratio
150		NPU28315	Erythrocytes(Blood)—Haemoglobin; mass concentration = ? g/L	Ercs(B)—Haemoglobin; mass c. = ? g/L	Erythrocytes	Blood		Haemoglobin		mass concentration		g/L	Clinical Biochemistry	Ratio
151	CCP	NPU19947	Plasma—Cyclic citrullinated peptide antibody(IgG); arbitrary substance concentration(procedure) = ? (p.d.u.)	P—Cyclic citrullinated peptide antibody(IgG); arb. subst.c.(proc.) = ? (p.d.u.)	Plasma			Cyclic citrullinated peptide antibody	IgG	arbitrary substance concentration	procedure	(p.d.u.)	Clinical Biochemistry	Ratio
152		NPU24819	Urine—3,4-Methylenedioxyamfetamine; mass concentration = ? µg/L	U—3,4-Methylenedioxyamfetamine; mass c. = ? µg/L	Urine		3,4-	Methylenedioxyamfetamine		mass concentration		µg/L	Clinical Pharmacology	Ratio
153		NPU04708	Blood—Plasmocytes; number concentration = ? × 109/L	B—Plasmocytes; num.c. = ? × 10⁹/L	Blood			Plasmocytes		number concentration		× 109/L	Clinical Biochemistry	Ratio
154		NPU24821	Urine—3,4-Methylenedioxymetamfetamine; mass concentration = ? µg/L	U—3,4-Methylenedioxymetamfetamine; mass c. = ? µg/L	Urine		3,4-	Methylenedioxymetamfetamine		mass concentration		µg/L	Clinical Pharmacology	Ratio
155	LH	NPU02618	Plasma—Lutropin; arbitrary substance concentration(IS 80/552; procedure) = ? IU/L	P—Lutropin; arb.subst.c.(IS 80/552; proc.) = ? IU/L	Plasma			Lutropin		arbitrary substance concentration	IS 80/552; procedure	IU/L	Clinical Biochemistry	Ratio
156		NPU19768	Plasma—Fibrinogen; mass concentration(coagulation;procedure) = ? g/L	P—Fibrinogen; mass c.(coag.; proc.) = ? g/L	Plasma			Fibrinogen		mass concentration	coagulation; procedure	g/L	Trombosis and Haemostasis	Ratio
157		NPU54291	Urine—Ritalinic acid; mass concentration = ? µg/L	U—Ritalinic acid; mass c. = ? µg/L	Urine			Ritalinic acid		mass concentration		µg/L	Clinical Pharmacology	Ratio
158	C-peptide	NPU04149	Plasma(fasting Patient)—Proinsulin C-peptide; substance concentration = ? nmol/L	P(fPt)—Proinsulin C-peptide; subst.c. = ? nmol/L	Plasma	fasting Patient		Proinsulin C-peptide		substance concentration		nmol/L	Clinical Biochemistry	Ratio
159	Anti-Tgase	NPU14566	Plasma—Transglutaminase antibody(IgA); arbitrary substance concentration(procedure) = ? (p.d.u.)	P—Transglutaminase antibody(IgA); arb. subst.c.(proc.) = ? (p.d.u.)	Plasma			Transglutaminase antibody	IgA	arbitrary substance concentration	procedure	(p.d.u.)	Clinical Biochemistry	Ratio
160		NPU24781	Urine—Methadone; mass concentration = ? µg/L	U—Methadone; mass c. = ? µg/L	Urine			Methadone		mass concentration		µg/L	Clinical Pharmacology	Ratio
161	Calcium ion	NPU01446	Plasma—Calcium ion(free); substance concentration = ? mmol/L	P—Calcium ion(free); subst.c. = ? mmol/L	Plasma			Calcium ion	free	substance concentration		mmol/L	Clinical Biochemistry	Ratio
162		NPU23591	Urine—Codeine; mass concentration = ? µg/L	U—Codeine; mass c. = ? µg/L	Urine			Codeine		mass concentration		µg/L	Clinical Pharmacology	Ratio
163		NPU03958	Urine—Protein; mass concentration = ? g/L	U—Protein; mass c. = ? g/L	Urine			Protein		mass concentration		g/L	Clinical Biochemistry	Ratio
164		NPU23881	Urine—Ethylmorphine; mass concentration = ? µg/L	U—Ethylmorphine; mass c. = ? µg/L	Urine			Ethylmorphine		mass concentration		µg/L	Clinical Pharmacology	Ratio

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165		NPU03695	Patient—Urine; volume(procedure) = ? mL	Pt—Urine; vol.(proc.) = ? mL	Patient			Urine		volume	procedure	mL	Clinical Biochemistry	Ratio
166		NPU28000	Urine—Oxycodone; mass concentration = ? µg/L	U—Oxycodone; mass c. = ? µg/L	Urine			Oxycodone		mass concentration		µg/L	Clinical Pharmacology	Ratio
167	Ret-Hb	NPU17007	Reticulocytes(Blood)—Haemoglobin(Fe); entitic amount-of-substance = ? fmol	Rtcs(B)—Haemoglobin(Fe); entitic am.s. = ? fmol	Reticulocytes	Blood		Haemoglobin	Fe	entitic amount-of-substance		fmol	Clinical Biochemistry	Ratio
168		NPU27388	Urine—Tramadol; mass concentration = ? µg/L	U—Tramadol; mass c. = ? µg/L	Urine			Tramadol		mass concentration		µg/L	Clinical Pharmacology	Ratio
169	HCO3	NPU02409	Plasma(Arterial blood)—Hydrogen carbonate; substance concentration(actual; 37 °C) = ? mmol/L	P(aB)—Hydrogen carbonate; subst.c.(actual; 37 °C) = ? mmol/L	Plasma	Arterial blood		Hydrogen carbonate		substance concentration	actual; 37 °C	mmol/L	Clinical Biochemistry	Ratio
170		NPU53120	Urine—Fentanyl; mass concentration = ? µg/L	U—Fentanyl; mass c. = ? µg/L	Urine			Fentanyl		mass concentration		µg/L	Clinical Pharmacology	Ratio
171	Ca125	NPU01448	Plasma—Cancer antigen 125; arbitrary substance concentration(procedure) = ? (p.d.u.)	P—Cancer antigen 125; arb.subst.c.(proc.) = ? (p.d.u.)	Plasma			Cancer antigen 125		arbitrary substance concentration	procedure	(p.d.u.)	Clinical Biochemistry	Ratio
172	CK	NPU22281	Plasma—Creatine kinase; catalytic concentration(IFCC 2002) = ? µkat/L	P—Creatine kinase; cat.c.(IFCC 2002) = ? µkat/L	Plasma			Creatine kinase		catalytic concentration	IFCC 2002	µkat/L	Clinical Biochemistry	Ratio
173	ESR	NPU17589	Blood—Sedimentation reaction; arbitrary length(procedure) = ? (p.d.u.)	B—Sedimentation reaction; arbitrary length(proc.) = ? (p.d.u.)	Blood			Sedimentation reaction		arbitrary length	procedure	(p.d.u.)	Clinical Biochemistry	Ratio
174		NPU28402	Plasma—Connective tissue disease related antibody; arbitrary substance concentration(procedure) = ? (p.d.u.)	P—Connective tissue disease related antibody; arb.subst.c.(proc.) = ? (p.d.u.)	Plasma			Connective tissue disease related antibody		arbitrary substance concentration	procedure	(p.d.u.)	Clinical Immunology	Ratio
175		NPU53097	Urine—Zopiclone; mass concentration = ? µg/L	U—Zopiclone; mass c. = ? µg/L	Urine			Zopiclone		mass concentration		µg/L	Clinical Pharmacology	Ratio
176		NPU18247	Plasma—Prolactin; arbitrary substance concentration(IS 84/500; procedure) = ? × 10-3 IU/L	P—Prolactin; arb. subst.c.(IS 84/500; proc.) = ? × 10⁻³ IU/L	Plasma			Prolactin		arbitrary substance concentration	IS 84/500; procedure	× 10-3 IU/L	Clinical Biochemistry	Ratio
177		NPU22299	Plasma—Apolipoprotein B; mass concentration = ? g/L	P—Apolipoprotein B; mass c. = ? g/L	Plasma			Apolipoprotein B		mass concentration		g/L	Clinical Biochemistry	Ratio
178		NPU53093	Urine—Zolpidem; mass concentration = ? µg/L	U—Zolpidem; mass c. = ? µg/L	Urine			Zolpidem		mass concentration		µg/L	Clinical Pharmacology	Ratio
179	INR	NPU01685	Plasma—Coagulation, tissue factor-induced; relative time(actual/norm; INR; IRP 67/40;procedure) = ?	P—Coagulation, tissue factor-induced; rel.time(actual/norm; INR; IRP 67/40; proc.) = ?	Plasma			Coagulation, tissue factor-induced		relative time	actual/norm; INR;IRP 67/40; procedure		Trombosis and Haemostasis	Ratio
180		NPU01972	Plasma—Estradiol; substance concentration = ? nmol/L	P—Estradiol; subst.c. = ? nmol/L	Plasma			Estradiol		substance concentration		nmol/L	Clinical Biochemistry	Ratio

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181		NPU03543	Plasma—Testosterone; substance concentration = ? nmol/L	P—Testosterone; subst.c. = ? nmol/L	Plasma			Testosterone		substance concentration		nmol/L	Clinical Biochemistry	Ratio
182		NPU19695	Plasma—Apolipoprotein A1; mass concentration = ? g/L	P—Apolipoprotein A1; mass c. = ? g/L	Plasma			Apolipoprotein A1		mass concentration		g/L	Clinical Biochemistry	Ratio
183		NPU04166	Urine—Acetoacetate; substance concentration = ? mmol/L	U—Acetoacetate; subst.c. = ? mmol/L	Urine			Acetoacetate		substance concentration		mmol/L	Clinical Biochemistry	Ratio
184	pCO2	NPU12481	Plasma(cord Blood)—Carbon dioxide; tension(37 °C) = ? kPa	P(cB)—Carbon dioxide; tension(37 °C) = ? kPa	Plasma	cord Blood		Carbon dioxide		tension	37 °C	kPa	Clinical Biochemistry	Ratio
185		NPU09226	Prostata specific antigen(Plasma)—Prostata specific antigen(free); mass fraction = ?	Prostata specific antigen(P)—Prostata specific antigen(free); mass fr. = ?	Prostata specific antigen	Plasma		Prostata specific antigen	free	mass fraction			Clinical Biochemistry	Ratio
186		NPU13041	Plasma—Birch antibody(IgE); arbitrary substance concentration(t3;procedure) = ? (p.d.u.)	P—Birch antibody(IgE); arb.subst.c.(t3; proc.) = ? (p.d.u.)	Plasma			Birch antibody	IgE	arbitrary substance concentration	t3; procedure	(p.d.u.)	Clinical Allergology	Ratio
187		NPU27315	Plasma—Inhalation antigen antibody(IgE); arbitrary substance concentration(IRP 75/502;(t3; g6; w6; e1; e5; d1; e3; m2; d2; t9; w19); procedure) = ? × 10^3 IU/L	P—Inhalation antigen antibody(IgE); arb.subst.c.(IRP 75/502; (t3; g6; w6; e1; e5; d1; e3; m2; d2; t9; w19); proc.) = ? × 10^3 IU/L	Plasma			Inhalation antigen antibody	IgE	arbitrary substance concentration	IRP 75/502; (t3; g6; w6; e1; e5; d1; e3; m2; d2; t9; w19); procedure	× 10^3 IU/L	Clinical Allergology	Ratio
188		NPU02195	Plasma(venous Blood;fasting Patient)—Glucose; substance concentration = ? mmol/L	P(vB; fPt)—Glucose; subst.c. = ? mmol/L	Plasma	venous Blood; fasting Patient		Glucose		substance concentration		mmol/L	Clinical Biochemistry	Ratio
189		NPU13098	Plasma—Timothy grass antibody(IgE); arbitrary substance concentration(g6;procedure) = ? (p.d.u.)	P—Timothy grass antibody(IgE); arb.subst.c.(g6; proc.) = ? (p.d.u.)	Plasma			Timothy grass antibody	IgE	arbitrary substance concentration	g6; procedure	(p.d.u.)	Clinical Allergology	Ratio
190		NPU18631	Urine—Bacterium; arbitrary number(procedure) = ? (p.d.u.)	U—Bacterium; arb.num.(proc.) = ? (p.d.u.)	Urine			Bacterium		arbitrary number	procedure	(p.d.u.)	Clinical Microbiology	Ratio
191		NPU21531	Plasma(Venous blood)—Glucose; substance concentration = ? mmol/L	P(vB)—Glucose; subst.c. = ? mmol/L	Plasma	Venous blood		Glucose		substance concentration		mmol/L	Clinical Biochemistry	Ratio
192		NPU13135	Plasma—Mugwort antibody(IgE); arbitrary substance concentration(w6;procedure) = ? (p.d.u.)	P—Mugwort antibody(IgE); arb.subst.c.(w6; proc.) = ? (p.d.u.)	Plasma			Mugwort antibody	IgE	arbitrary substance concentration	w6; procedure	(p.d.u.)	Clinical Allergology	Ratio
193		NPU53974	Plasma—Amylase; catalytic concentration(37 °C; procedure) = ? U/L	P—Amylase; cat.c.(37 °C; proc.) = ? U/L	Plasma			Amylase		catalytic concentration	37 °C; procedure	U/L	Clinical Biochemistry	Ratio
194		NPU04146	Plasma—Cholesterol+ester, in LDL/Cholesterol+ester, in HDL; substance ratio = ?	P—Cholesterol+ester, in LDL/Cholesterol+ester, in HDL; subst.ratio = ?	Plasma			Cholesterol +ester, in LDL/ Cholesterol +ester, in HDL		substance ratio			Clinical Biochemistry	Ratio

Rank (see commentary)	*Non-authorized indications (trivial terms and abbreviations)	**NPU identifier	Comprehensive, systematic NPU term of laboratory examinations	Abbreviated NPU term of laboratory examinations	System	Sys-spec.	Prefix	Component	Comp-spec.	Kind-of-property"	Procedure	Unit	Speciality	Scale type
195	TPO	NPU12229	Plasma—Thyroid peroxidase antibody; arbitrary substance concentration(procedure) = ? (p.d.u.)	P—Thyroid peroxidase antibody; arb.subst.c.(proc.) = ? (p.d.u.)	Plasma			Thyroid peroxidase antibody		arbitrary substance concentration	procedure	(p.d.u.)	Clinical Biochemistry	Ratio
196	52 kDa Ro protein antibody	NPU18242	Plasma—E3 ubiquitin-protein ligase TRIM21 antibody(IgG); arbitrary substance concentration (procedure) = ? (p.d.u.)	P—E3 ubiquitin-protein ligase TRIM21 antibody(IgG); arb.subst.c.(proc.) = ? (p.d.u.)	Plasma			E3 ubiquitin-protein ligase TRIM21 antibody	IgG	arbitrary substance concentration	procedure	(p.d.u.)	Clinical Immunology	Ratio
197	hCG beta chain	NPU01580	Plasma—Choriogonadotropin beta-chain; arbitrary substance concentration(IRP 75/551; procedure) = ? IU/L	P—Choriogonadotropin beta-chain; arb.subst.c.(IRP 75/551; proc.) = ? IU/L	Plasma			Choriogo-nadotropin beta-chain		arbitrary substance concentration	IRP 75/551; procedure	IU/L	Clinical Biochemistry	Ratio
198		NPU04153	Leukocytes(Blood)—Large unstained cells; number fraction = ?	Lkcs(B)—Large unstained cells; num.fr. = ?	Leuko-cytes	Blood		Large un-stained cells		number fraction			Clinical Biochemistry	Ratio
199	FSH	NPU18869	Plasma—Follitropin; arbitrary substance concentration (procedure) = ? (p.d.u.)	P—Follitropin; arb.subst.c.(proc.) = ? (p.d.u.)	Plasma			Follitropin		arbitrary substance concentration	procedure	(p.d.u.)	Clinical Biochemistry	Ratio
200		NPU13227	Plasma—Cat dander-epithelium antibody(IgE); arbitrary substance concentration(e1; procedure) = ? (p.d.u.)	P—Cat dander-epithelium antibody(IgE); arb.subst.c.(e1; proc.) = ? (p.d.u.)	Plasma			Cat dander-epithelium antibody	IgE	arbitrary substance concentration	e1; procedure	(p.d.u.)	Clinical Allergology	Ratio
201	CEA	NPU19719	Plasma—Carcinoembryonic antigen; mass concentration = ? µg/L	P—Carcinoembryonic antigen; mass c. = ? µg/L	Plasma			Carcino-embryonic antigen		mass concentration		µg/L	Clinical Biochemistry	Ratio

* '1' indicates the most frequent laboratory examination perfromed by Danish, Dutch, Norwegian and Swedish laboratories

** The content of this column has not been validated, and may solely be a help for the readers to find the exact laboratory examination. The trivial terms may vary between languages and cultures.j

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Evaluation of visual serum indices measurements and potential false result risks in routine clinical chemistry tests in Addis Ababa, Ethiopia

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ABSTRACT

Background:

Serum indices (SI) including hemolyzed, lipemic, and icteric samples, affects the accuracy of test result. The aim of this study was to evaluate SI values done by visual inspections and potential false result risks by comparing with actual measurements done by Cobas 6000 Chemistry analyzer at Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia.

Methods:

An observational, cross-sectional study was conducted from April to May 2017 on samples referred to Clinical Chemistry laboratory of EPHI, Ethiopia. These samples SI values, after visual inspection by three trained observers, was analyzed again on Roche Cobas 6000 analyzer (RCA). The generated data was analyzed by using weighted kappa methods on STAT statistical software version 20.

Results:

From a total of 1509 samples, SI values identified by the RCA as hemolysis, icteric, and lipemic were 933 (62%), 74(5%) and 59(4%) respectively. The SI average weighted kappa between RCA and visual inspection were: 0.1870, 0.3421, and 0.1259 for hemolysis, icteric, and lipemic samples, respectively. Combined inter-observers variability among observers for hemolysis, Icterus, and lipemic samples were 0.4758, 0.3258, and 0.3628 respectively. The best agreement among observers was in the case of hemolysis (0 grades), while the lowest agreement was observed in the case of icterus (+3 grades). In addition, test parameters, such as CK-MB (22%), and LDH (20%) were falsely accepted, whereas Cl^- and Na^+ (up to 25%) were falsely rejected tests by observers. On the other hand, results rejected by Cobas SI assessments included CK-MB (22%), LDH (20%), and BIL-D (4%).

Conclusion:

Visual inspection of SI showed poorly agreement with automated system. Thus, there is genuine need for more training of Laboratory professionals on identification of SI, and as much as possible SI should be done by automated system to improve quality of test results.

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Abbreviations

***AAU**: Addis Ababa University*

***ALT:** Alanine Amino Transferase*

***AST:** Aspartat Amino Transferase*

***CCH:** Clinical Chemistry*

***CK-MB:** Creatine Kinase*

***EPHI:** Ethiopian Public Health Institute*

***LDH:** Lactate Dehydrogenase*

***RPM:** Revolution Per Minute*

***SI:** Serum Indices*

***SMLS**: School of Medical Laboratory Sciences*

***SOPS:** Standard Operating Procedures*

***UIBC:** Unsaturated Iron Binding Capacity*

***HIL**: Hemolysis, Icterus, Lipemia*

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BACKGROUND

In clinical laboratory activities, 68-77 percent of errors occur in the pre-analytical phase [1, 2]. Efficient management and monitoring of the pre-analytical sources of interference is critical to the quality of clinical laboratory analytical process and to the quality of patient results. Clinical laboratory errors can lead to incorrect results dispatched to physicians that result in erroneous patient laboratory report interpretation and conclusion. This in turn highly affects the whole healthcare system [3]. Among the main causes of pre-analytical error, serum indices (SI) which includes Hemolysis, Icterus and Lipemia (HIL) are the leading ones.

Hemolysis is one of the major causes of pre-analytical source of error. It accounts for 40% to 70% of unsuitable samples [4]. Hemolyzed samples (>95 percent) are attributable to in vitro processes resulting from inappropriate sample collection technique or transport [5]. The hemolyzed sample affect different clinical tests by mechanism of leakage of constituents of red blood cells into plasma or serum, spectrophotometric/colorimetric interference by hemoglobin, participation of the hemoglobin in the reaction through inhibition, and dilution of serum or plasma components [6].

Lipemia, the other cause of pre analytical errors, results from increased concentration of triglyceride-rich lipoproteins in blood. This lipemic serum causes cloudy/turbid appearance of serum or plasma. Lipemic sample test interference is associated with light scattering effects, and may

increase absorbance during end point reactions and non-blanked reactions for some analytes. In addition, lipemia is associated with volume displacement effect, and greatly decreases the value of some analytes [7].

Icterus, another main cause of pre-analytical errors, result from diseases associated with increased bilirubin production or inappropriate bilirubin excretion. Icterus samples interfere in laboratory tests by direct interaction with different test analytes or reagents resulting in decreased analyte values, and creating spectral interferences during color measurement [6, 8].

Different studies have showed that visual assessments have limitations, including subjectivity, difficult in identification by the naked eye, time consumption, and inability to inspect by naked eye when the sample is covered by multiple barcodes.

In Ethiopia medical laboratories, common interferences are usually determined by using visual assessments. But up to the knowledge of this study groups, there is no study conducted on comparison of serum indices value against visual inspection of the samples.

Thus, the aim of this study was to compare Serum Indices value measurements and visual assessment using Cobas 6000 chemistry analyzer, and identify potential false result risks at the routine clinical chemistry laboratory at Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia.

MATERIALS AND METHODS

This research was observational, cross-sectional study, conducted from April to May 2017 at EPHI Clinical Chemistry Laboratory, Addis Ababa, Ethiopia. All patient samples referred to EPHI Clinical chemistry department, during the study periods, were used as a source of samples. During actual study, all referred samples except those that were unlabeled and with insufficient volume, were utilized. Accordingly, a total of 1509 samples were analyzed visually and using serum indices (Roche Serum Indices Gen 2). Visual inspection was done by laboratory technologists who took intensive training for three days from experienced laboratory technologist to identify interferences. In addition, to standardize the visual assessment, colored photo and categories of HIL in serum or plasma grading were prepared (Table 1).

Table 1 Categorization of HIL indices with grading*

Grading	Hemolysis SI indices value, hemoglobin, mg/dl	Icteric SI indices value, bilirubin in mg/dl	Lipemic SI indices value, intralipid, mg/dl
0	<9	<2.5	<40
+1	10-199	2.5-4.9	40-99
+2	200-299	5.0-9.9	100-199
+3	300-399	10-119.9	200-299
+4	>400	>40	>300

*SI=Serum indices; *adapted from Lim et al [9].*

Laboratory analysis

Blood samples (3-5 ml) without anticoagulant were collected from each patients, and centrifuged at 2500 revolution per minute (RPM) for 5 minutes according to EPHI clinical chemistry standard operating procedure (SOP). Then the separated serum samples were inspected visually by three laboratory technologists who participated in the study. Visual inspection was performed with grading according to standardized colored photos and a consensus was reached when doubtful samples were interpreted according to these photos.

Those samples which were evaluated visually were analyzed again for serum indices using Roche serum indices of Roche Gen 2 without delay by Cobas 6000 (Roche Diagnostics, Mannheim, Germany). In addition, the Cobas 6000 instrument was used to perform 22 different routine clinical chemistry tests (as requested by the physicians), and assessed the degree of interfaces on the test parameters.

Description of Roche serum indices

The Serum Indices Gen. 2 assay is based on calculations of absorbance measurements of diluted samples at different dichromatic wavelength pairs to provide a semi-quantitative representation of levels of lipemia, hemolysis and icterus present in serum and plasma samples. The analyzers take an aliquot of the patient specimen and dilute it in saline solution (0.9 % sodium chloride) to measure the absorbance for lipemia at 660 nm (primary wavelength) and 700 nm (secondary wavelength), for hemolysis at 570 nm (primary wavelength) and 600 nm (secondary wavelength), and for icterus at 480 nm (primary wavelength) and 505 nm (secondary wavelength). From these absorbance values the instrument calculates the SI [10].

Quality control and quality assurance

Before running patient samples, two levels of quality control materials were run to assess the functionality of the instrument and test procedures. In addition, well-trained and experienced laboratory professionals participated in the analysis procedure.

Data management and statistical analysis

The statistical analyses were performed by STATA version 14. Prior to analyses, the entered data were cross-checked against the original paper data collection form. Agreement between serum indices and observers were assesses by weighted kappa. Interpretation of kappa coefficient was as follows: - <0 = Less than chance agreement, 0.00-0.20 = Slight agreement, 0.21-0.40 = Fair agreement, 0.41-0.60 = Moderate agreement, 0.61-0.80 = Substantial agreement, 0.81-1.00 = Almost perfect agreement [11].

Ethical consideration

Before the research work, ethical clearance was obtained from the School of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. In addition, the project was presented to EPHI scientific and Ethical Review Office (SERO) and got additional ethical approval. In order to protect patient confidentiality patient identifiers like name and telephone number were not collected. Moreover, Patient's registration (sample ID) coding system and patient detail information's were secured.

RESULTS

Comparison of visual inspection and serum indices of Roche for hemolysis among observers

From a total of 1509 samples, cobas automated SI measurement revealed that 933 (62%) were hemolyzed. These values when assessed by visual inspection, observer one, two and three

recognized 257 (17%), 343 (23%) and 336 (22%) samples, respectively. The weighted kappa between observers and serum indices was 0.1709 for observer 1, 0.1764 for observer 2, and 0.2136 for observer 3. Accordingly, there was slight agreement with Cobas by observer 1 and 2, and fair agreement between observer 3 and serum indices, as shown in Table 2.

Comparison of visual inspection and serum indices of Roche for Icterus among observers

From a total of 1509 samples, automated serum indices revealed that 74 (5%) were icteric. Meanwhile, when icterus assessed by Visual inspection, observer one, two and three reported 158 (11%), 76 (5%) and 81 (5.4%) samples, respectively. The weighted kappa shows fair agreement for observer one, and moderate agreement for observer two and three, with Kappa values of 0.2682, 0.4136, and 0.3445, respectively, as shown in Table 3.

Comparison of visual inspection and serum indices of Roche for lipemia among observers

From a total of 1509 samples, Cobas 6000 SI value reveled 59 (4%) as lipemic. Meanwhile, observer one, two and three upon visual assessment identified 207 (14%), 208 (14%) and 148 (10%) samples, respectively as lipemic. The weighted k coefficient was 0.1169 for observer 1, 0.1221 for observer 2, and 0.1386 for observer 3 with slight agreement between serum indices and visual inspection for all observers, see Table 4.

Inter-observers variability for visual inspection among observers

In the present study, agreement among Inter-observers variability was assessed. Accordingly, the best overall agreement among observers was in the assessment of hemolysis (0 grade) with the kappa value of 0.6600 and the lowest degree of agreement was observed in assessing icterus (+3 grade) with kappa value 0.1016, as shown in Table 5.

Potential risk introduced by observers

One of the objectives of this study was to assess risk of false result delivery following poor visual SI evaluations. Accordingly, test parameters which were falsely accepted by visual observers while rejected by Cobas SI assessments included CK-MB (22%), LDH (20%), and BIL-D (4%), as shown in Figure 5.

On the other hand, routine clinical chemistry tests which were falsely rejected by visual observers while accepted by cobas serum indices analysis, included Cl^- and Na^+ (n=178, 25%), and BIL-T (n=17, 7%), as shown in Figure 2.

DISCUSSION

Efficient laboratory service is the cornerstone of modern health care systems. In this regard, mainly in the clinical chemistry areas, scientific innovations contributed a lot to substantial improvements in reducing laboratory diagnostic errors. Nevertheless, shortage of advanced clinical chemistry instruments, affordability of instrument running costs, along with shortage of experienced laboratory professionals are still a challenge in most developing countries to produce quality laboratory results.

In the present study, out of the 1509 specimens submitted to EPHI for clinical chemistry tests, hemolysis was detected in 933 (62%) samples. For hemolysis, visual inspection showed a fair agreement with automated detection, at a kappa value of less than 0.21 for observers. A similar study performed by Giuseppe L. et al, compared detection of hemolysis in 800 serum samples, where 8% of samples were hemolytic and the automation versus visual inspection difference showed a weighted kappa value of 0.42. Hemolysis was overestimated using visual assessment of serum samples and

Table 2 Comparison of visual inspection and serum indices of Roche for hemolysis, EPHI, Ethiopia, 2017

	Serum Indices	Visual Inspection of hemolysis							
		0	+1	+2	+3	+4	Total	Level of agreement	Kappa
Observer 1	0	576	8	0	0	1	576	48.97%	0.1709
	+1	667	168	57	21	4	917		
	+2	0	2	2	2	2	8		
	+3	0	1	3	0	1	5		
	+4	0	0	1	0	2	3		
	Total	1234	179	63	23	10	1509		
Observer 2	0	517	9	3	2	1	576	47.05%	0.1764
	+1	604	143	98	52	20	917		
	+2	0	1	1	3	3	8		
	+3	1	0	1	2	1	5		
	+4	0	0	0	0	3	3		
	Total	1166	153	103	59	28	1509		
Observer 3	0	565	8	2	0	1	576	51.6%	0.2136
	+1	607	208	78	13	11	917		
	+2	0	1	3	0	4	8		
	+3	0	1	2	1	1	5		
	+4	1	0	0	0	2	6		
	Total	1173	218	85	14	19	1506		

Table 3 Comparison of visual inspection and serum indices of Roche for icterus, EPHI, Ethiopia, 2017

	Serum Indices	Visual inspection of hemolysis							
		0	+1	+2	+3	+4	Total	Level of agreement	Kappa
Observer 1	0	1327	80	24	3	1	1435	89.26%	0.2682
	+1	11	9	5	4	2	31		
	+2	9	6	8	3	7	33		
	+3	4	0	2	0	1	7		
	+4	0	0	0	0	3	3		
	Total	1351	95	39	10	14	1509		
Observer 2	0	1404	19	12	0	0	1435	94.37%	0.4136
	+1	14	9	7	1	0	31		
	+2	10	3	9	7	4	33		
	+3	4	0	1	1	1	7		
	+4	1	0	0	1	1	3		
	Total	1433	31	29	10	6	1509		
Observer 3	0	1392	35	7	0	1	1435	93.51%	0.3445
	+1	15	9	6	1	0	31		
	+2	15	4	8	6	0	33		
	+3	5	1	0	1	0	7		
	+4	1	1	0	0	1	3		
	Total	1428	50	21	8	2	1509		

Table 4 Comparison of visual inspection and serum indices of Roche for lipemia, EPHI, Ethiopia, 2017

	Serum Indices	Visual inspection of hemolysis							
		0	+1	+2	+3	+4	Total	Level of agreement	Kappa
Observer 1	0	1280	81	63	21	5	1450	85.15%	0.1169
	+1	22	4	13	8	7	54		
	+2	0	1	0	0	4	5		
	+3	0	0	0	0	0	0		
	+4	0	0	0	0	0	0		
	Total	1302	85	76	29	16	1509		
Observer 2	0	1279	123	41	6	1	1450	81.22%	0.1221
	+1	20	7	18	8	1	54		
	+2	2	0	0	2	1	5		
	+3	0	0	0	0	0	0		
	+4	0	0	0	0	0	0		
	Total	1301	130	59	16	3	1509		
Observer 3	0	1332	97	18	2	1	1450	88.73%	0.1386
	+1	29	7	10	3	5	54		
	+2	0	1	0	1	3	5		
	+3	0	0	0	0	0	0		
	+4	0	0	0	0	0	0		
	Total	1361	105	28	6	9	1509		

Table 5 Inter-observers variability among different technologist, EPHI, Ethiopia, 2017

Interferences		Visual grading value					
		0	+1	+2	+3	+4	Combined
Kappa Value	Hemolysis	0.6600	0.3563	0.2619	0.1486	0.4847	0.4758
	Icterus	0.4643	0.1310	0.3238	0.1016	0.2692	0.3258
	Lipemic	0.5022	0.2265	0.2489	0.2067	0.2812	0.3628

Figure 1 Total number of tests performed and the number of tests falsely accepted by observers, EPHI, Ethiopia, 2017

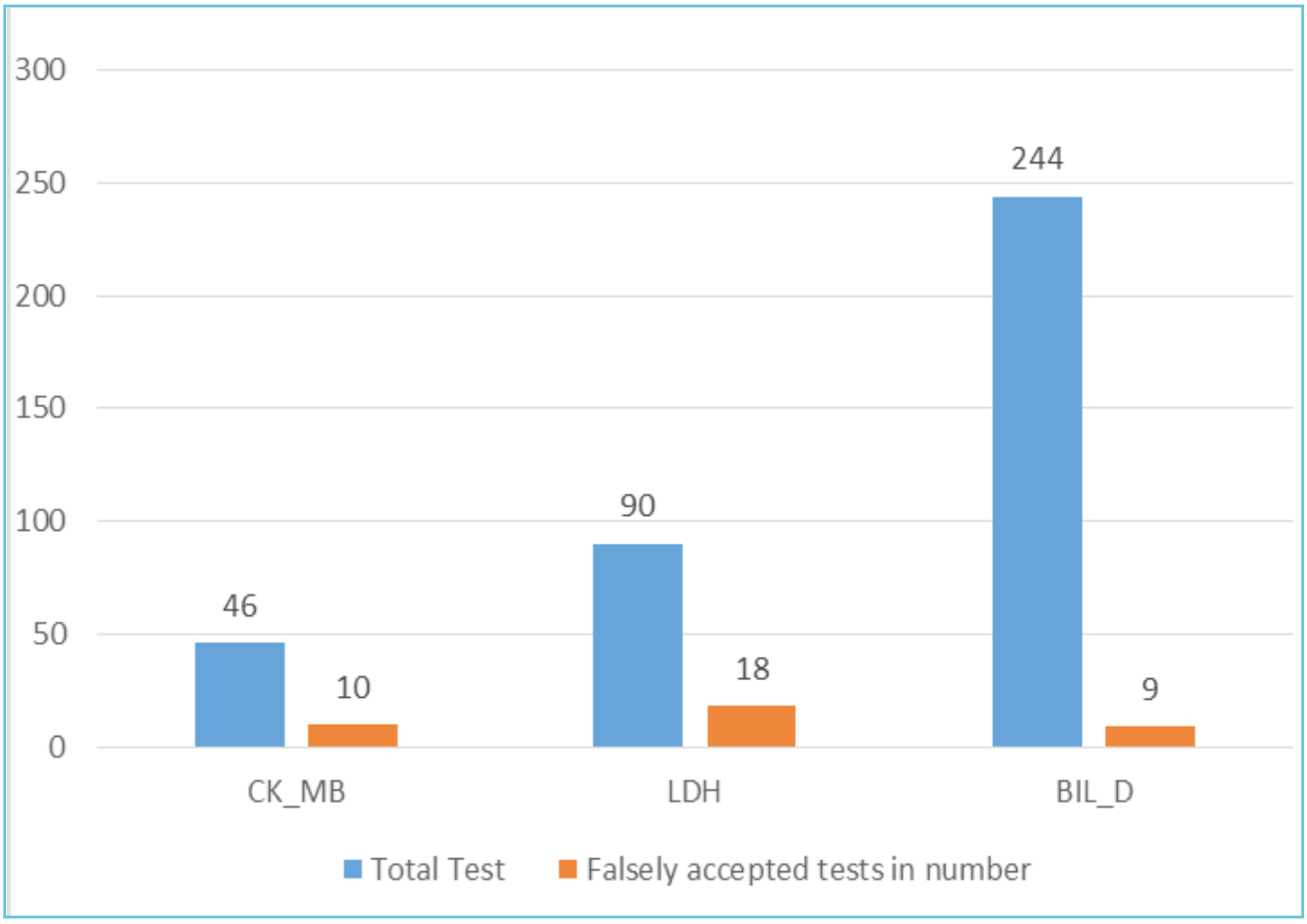
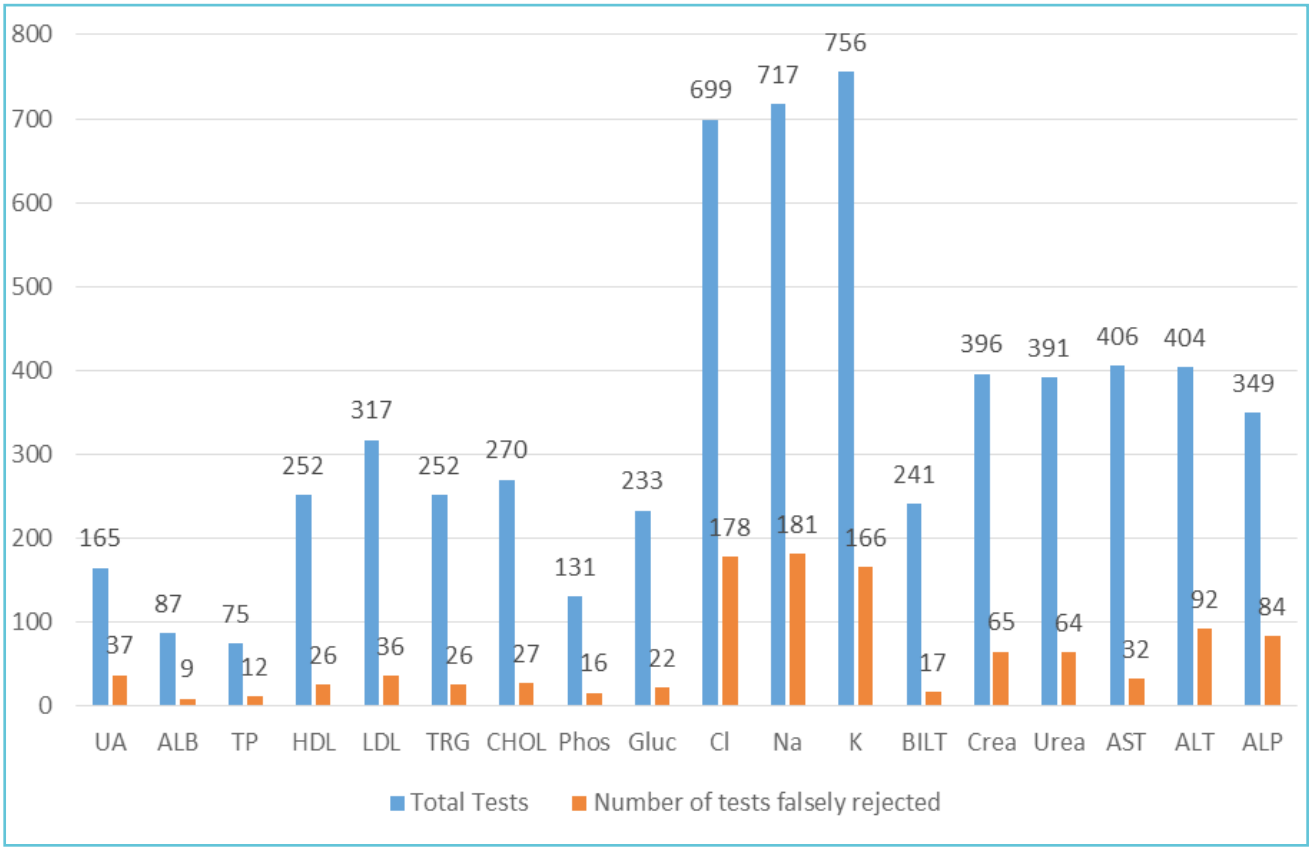


Figure 2 Total number of tests performed and the number of falsely rejected tests by observers, EPHI, Ethiopia, 2017



underestimated in plasma samples [5]. Studies indicated that following damage to red cell membrane and a resultant hemoglobin concentration greater than 0.3g/l, hemolysis can be recognized by the naked eye [12]. But the visual inspection of hemolysis varies from person to person due to factors, including differences in laboratory work experiences, individual ability to differentiate color intensity, and on job training opportunities.

Hemolysis affects result of different test parameters. In the present study, our observers falsely accepted a number of samples that were (+1) hemolytic as per the automated approach. If the tests were run just by visual inspection, test parameters including CK-MB, DBIL, LDH, AST, UIBC, TBIL and K^+ were labeled as false laboratory results. Similar study done by Jeffery et al. indicated that use of the automated hemolysis indices is highly recommended and that potassium in neonatal and adult specimens should be reported with a correction formula, since it might be beneficial to the clinical management of the patient [13]. The most probable cause of poor identification of hemolytic samples by visual inspection might be due to poor knowledge and lack of observer experience on hemolysis. In addition differences on sensitivity of the naked eye as compared to spectrophotometers could be another reasoning.

Another finding of the present study was that the automated approach identified a total of 74 (5%) samples with icterus; whereas the icterus indices recorded by observer 1, 2, and 3 were 158 (11%), 76 (5%) and 81 (5.4%), respectively. Upon statistical analysis, kappa value agreement

between the automated machine and the three visual observers were 0.3421, i.e., fair agreement. A similar study conducted in Croatia indicated that from the total of 1727 routine biochemistry samples, 101 samples were identified as icteric using visual inspection while automated serum indices detected only 74 samples, with moderate agreement between the two icterus indices detection approaches at weighted kappa values of 0.529 with moderate agreement [3].

The only parameter affected by grade icterus (+1) was the triglyceride assay. Similar findings were reported in a study conducted by Fatuma et al. on the study of assessment of serum indices implementation on cobas 6000. In this study, a total of 717 samples with no interferences by visual inspection were analyzed. From this, they found 102, 4 and 2 samples were hemolytic, lipemic and icteric, respectively [14].

In this present study a total of 207 (14%) lipemic samples were identified by the automated approach, and upon visual assessment observe one, observer two and observer three reported lipemia in 208 (14%), 148 (10%) and 59 (4%) samples, respectively. The average weighted kappa for the three observers was 0.1258, with slight agreement with the automated approach. The findings were similar to results reported by other researchers [3, 7]. Test results from lipemic samples may be inaccurate and can lead to medical errors, and as such represent a considerable risk to patient health [15, 16]. Studies indicate that lipemia is associated with diet and alcohol intake; as well as due to different pathological conditions including diabetes mellitus, hypertriglyceridemia, chronic renal failure and lupus erythematosus [17].

Studies showed that lipemic indices estimation ensures that the sample is fit for analysis. The use of automated lipemic estimation overcomes the limitations associated with visual estimation by providing a more objective and accurate estimate of lipemia [14, 18].

STRENGTH AND LIMITATION

Strength of this study include its large sample size, and to the best of authors' knowledge this study is the first of its kind in Ethiopia. However, there are certain limitations that need to be considered when interpreting our finding, since the numbers of observers and the analytes measured were limited.

CONCLUSION

Ethiopia and most other developing countries are now delivering quality laboratory services, and also apply for local/international laboratory accreditation. In this regard the present study demonstrates that visual inspection will introduce significant pre-analytical errors with regards to SI evaluations, and lead to false results. Thus, as a recommendation:

- Further studies are needed in the area in order to study the level of agreement between visual inspection and automated serum indices value for more specific parameters.
- Continued training on visual inspection for medical laboratory technologists in order to increase the potential of identifying interferences.
- Medical laboratories should be encouraged to implement automated serum indices measurement to detect interferences.

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Authors' contributions

TG, AA, FM, MS, AA, WH, ZG, FG: designed the study, monitored data collection, and prepared the manuscript.

MW, FC: Principle advisors of the study, and participated in conception and designing of the

study, and revised the manuscript critically for important intellectual content.

All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Ethical clearance and consent to participate

Ethical clearance for the study was obtained from the Department of Medical Laboratory Sciences, College of Health Sciences Addis Ababa University, Ethiopia.

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Best practices in the implementation of a point of care testing program: experience from a tertiary care hospital in a developing country

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ABSTRACT

Implementation of a structured Point of Care Test (POCT) program is challenging. Traditionally POCT was unregulated and the aim was to introduce a structured POCT program at our tertiary care hospital to ensure compliance with regulatory standards. The purpose of this article is to describe how a hospital in a developing country with limited resources has approached POCT program initiative. The benefits offered by such systems, including cost-effectiveness, robustness and the ability to generate reliable accurate POCT results in a short time, are appropriate to the clinical and social needs of the developing world.

BACKGROUND

Driven by quicker diagnostic paybacks, point of care testing (POCT), also known as bedside testing, near-patient testing, alternate-site testing, and ancillary testing, has modernized patient care (1). The College of American Pathologists (CAP) describes POCT as 'testing that does not require permanent dedicated space and it refers to those analytical patient-testing activities provided within the institution, but performed outside the physical facilities of the clinical laboratories' (2).

The POCT tests offer rapid results, allowing for timely initiation of appropriate management by reducing the turnaround time of the results through minimizing delays and errors in sample transport and processing (3, 4). Potential patient satisfaction comes from testing that is more convenient and less traumatic with minimal amount of blood draw. Furthermore improved turnaround time due to POCT results in prompt management reducing length of hospital stay (5, 6). Although the unit cost of POCT is higher than central laboratory, overall the testing is patient focused and cost-effective by reducing hospital stay and improving patients flow through busy critical care areas and emergency departments (7-9).

Despite the relative simplicity of POCT, regulations like Joint Commission International (JCI) and CAP dictate that all hospital based POCT must be supervised by the central laboratory. These regulatory bodies make certain that the laboratory director is responsible for standards of performance in all domains of POCT including pre-analytical, analytical and post analytical aspects. Delivery of medical diagnostic testing outside of the well-controlled environment of the clinical laboratory are affected by several organizational, environmental, operational, and technical challenges (10).

Managing preanalytical, analytical, and post-analytical processes compatible with centralized laboratory testing is a major challenge in POCT program implementation (11). Regular laboratory surveillance, inspection and prompt corrective action is essential for smooth operation of any POCT program. Additionally any POCT program requires continuous training of the operators, competency, method validation, and ongoing comparison with central laboratory results (12, 13). It is essential to maintain an appropriate record trail linking POCT operators' training and competency with POCT device validation, verification, maintenance and quality control. POCT execution and oversight poses a great challenge for laboratories as the testing being performed is actually out of their hands. Still the laboratory is responsible for ensuring that patient testing is performed in compliance with laboratory accreditation standards (14).

The POCT program at Aga Khan University Hospital (AKUH) was initiated in 2014 with the objectives of ensuring patient testing performed is in compliance to CLSI standards. Hereby we report our experience in establishment, organization and successive execution of POCT program at AKUH. The purpose of this review article is to highlight the importance of clinical needs assessment for development of POCT program, to discuss challenges in POCT implementation. We hereby provide a road map for POCT implementation and smooth execution.

NEEDS ASSESSMENT FOR POCT PROGRAM

Delineating program proposal

Like all laboratory testing, many regulatory guidelines have been put forth to address quality control, training and documentation to ensure patient safety. To start off with a thorough review of literature including International Organization for Standardization (ISO) guidelines, CLSI, Joint Commission International (JCI)

and CAP standards specific for POCT was completed by the Chemical Pathologists (15-17). A proposal delineating the scope of services was developed at the Section of Chemical Pathology, in the Department of Pathology and Laboratory Medicine, AKUH. It was decided that POCT would be performed in inpatient locations including wards, emergency department, operating rooms, special care units and intensive care units of the hospital. The POCT proposal highlighted the shortcomings of existing bedside testing identified in need assessment.

The core of the document was a regulatory proposal for the implementation of POCT including aspects related to personnel responsibilities, quality assurance, data management, and future tendencies. The goals of implementing the POCT program in the institute were clearly defined in the proposal.

The goals of POCT program were defined as follows:

- To ensure that POCT is high quality and cost-effective.
- To give guidance to all users and potential users of POCT.
- To provide consistency of test offering at all POCT sites.
- To simplify billing procedures on POCT sites.
- To provide faster turnaround times in test results with minimal inconvenience to the patient.
- To provide an organization-wide standardized policy for POCT application.

This document included following initial tasks as part of POCT program: outlining an organizational structure, defining roles and responsibilities of POCT teams and members and describing clinical needs of proposed POCT tests. The proposal was shared and approved by the senior management of the hospital and all stake holders.

Evidence based approach for POCT program development

Clinical needs assessment is a process by which information is gathered regarding the scope and potential impact of gaps or deficiencies in the current delivery and practice of health care (18). The POCT need assessment was done to gather information regarding current practices and clinical needs for developing a POCT program in our institute. Multiple surveys and site visits were conducted by the laboratory team at all the inpatient sites in the hospital prior to the development of the POCT program. The purpose of these surveys and site visits were to identify potential end users, optimize the use of the deployed equipment and identify the changes required to make the project efficient and effective by taking feedback from all concerned stakeholders. Table 1 outlines the domains which were covered in the surveys.

Cross sectional interview-based surveys and site visits were done at all the areas of the hospital where bedside testing was done. The survey included the infrastructure and connectivity requirements and current volumes of tests being performed near patients. The presence of deficiencies, gaps and challenges in POCT operation was documented (Table 2).

While most laboratory testing continued to be performed in the main clinical laboratory, arterial blood gases, electrolytes, glucose, urine analysis were performed in wards and critical care areas without central laboratory supervision, report generation, lack of training and lack of evidence of quality control. Manual record-keeping was inadequate for an audit trail for quality assurance. Compliance with requirements for documentation was difficult using handwritten records and going paperless was one of our goals. To address the challenges of POCT, to share experiences faced in previous clinical audits and to solicit opinions on executing POCT program

Table 1 Clinical needs assessment domains to identify and characterize existing gaps in current system

Personnel related	• Stakeholders assessment • User needs assessment • Acceptability assessment • Organization and responsibilities • Identification of POCT users
Policy and procedures	• POCT equipments and method validation requirement • Training and competency of users • Purchasing and inventory processes • Data management • Record keeping of POCT information • Quality control and proficiency testing • Security access
Facilities and safety	• Device location and POCT sites identification • Landscape analysis • Information technology connectivity check • Electrical points check

multiple focus group discussions with nursing managers were conducted and a strategic POCT roadmap was delineated.

POCT IMPLEMENTATION

Organizational structure

While the nursing staff and physicians may understand the day-to-day operation and provision of results, the overall responsibility of POCT program generally lies with the laboratory director. Responsibilities of the clinical laboratory include organization and implementation of the program, performing technical and general oversight and clinical consultancy and ensuring quality assurance. Laboratory director ensure compliance with all applicable regulations, rules and standards. To successfully achieve POCT implementation in an institute, a multidisciplinary organizational approach is a prerequisite. First and foremost, a clear organizational structure should be put in place for appropriate functioning and optimum utilization of each POCT site (19).

A multidisciplinary team comprising of all stakeholders with representatives from Pathology, Material and Management Division (MMD), IT, Biomedical Engineering (BE) and Nursing was formalized for execution of POCT program at AKUH. The team presented the POCT program at the Joint Staff meeting of the institute for approval. Concerns that arose with POCT implementation, like problems with ensuring quality, potential

Table 2 Deficiencies identified during clinical needs assessment

Categories	Gaps identified
Pre-analytical	• Lack of instructions for specimen collection and preservatives • Lack of evidence of training and competence assessment regarding pre-analytical factors that may influence the results • Lack of information regarding identification of POCT operator • POC test charging mechanism
Analytical	• Lack of evidence of training and competence assessment of POCT users • Lack of evidence of quality control processes • Absence of written quality assurance and quality control policies • No participation in proficiency testing program • Less than optimal information on policies, regulations, supplies & standard operating procedures • No identification or trail of site of testing • No records of equipment calibration and maintenance
Post analytical	• Absence of report generation • Manual recording of patient results • Previous POCT records of patients not accessible • Absence of records of POCT results interpretation • There is no connectivity of equipment/devices with Laboratory information system for prompt results reporting and to minimize errors

conflicts of interest, and an uncertainty of the responsibility, were all addressed with the stakeholders and the POCT end-users.

POCT policies and procedures

As per CLSI guidelines quality management system approach was followed for the development of standards and policies for POCT program. The organizational and regulatory requirements that should be considered when implementing POCT program in an institute should all be documented (Table 3). Laboratory director was made responsible for standards of performance in all areas, including quality control, quality assurance and test utilization in patient care. Each POCT site that performs POCT must have written policies and procedures available at the testing sites. Quality management plan, policies and testing procedures were written down and simultaneously POCT training program and curriculum were outlined by the pathologists and shared with POCT team members for approval and critique. Oversight and control of POCT program was hence provided by the laboratory

Table 3 Quality management system plan including policies and procedures needed for POCT program

POCT quality management system essentials	Quality management plan should include these policies/processes
Organization and integration	• POCT Committees • Roles and Responsibilities of teams • Responsibilities of POCT coordinator • Workflow • POCT program contact information
Quality assurance	• Internal quality control • Proficiency testing • Assessment and audits • Equipment procurement and method selection • Method validation • Standard operating procedures of each POCT • Result reporting and recording • Panic value reporting procedure • Unusual result reporting procedure • Equipment maintenance • Inventory and storage management • Safety and infection control • Occurrence management • Troubleshooting and backup plan • Customer satisfaction and complaint handling • Sample retention and storage process • Document control policy
Training and competence assessment	• Training curriculum • Training process • Competency assessment • Certification and access to POCT device

directorship along with all necessary assistance to run this program smoothly. The laboratory in writing agreed that under this program, a standard package of POCT services can be provided at any clinical facility in the institute, as long as the required training, proficiency testing, quality control and validation procedures were performed, verified and documented.

Coordination of central laboratory and POCT

The ultimate responsibility of POCT lies with the laboratory director. As soon as the POCT program approval was sought the central laboratory identified and selected a POCT coordinator from the clinical laboratory staff. The main role of POCT coordinator was to assist in oversight, management and synchronization of the entire POCT program. The responsibilities of POCT coordinator included all aspects of the POCT service, including overall supervision, management and oversight of POCT sites and devices (20). Additionally, he/ she would take input from and interface with the laboratory director regarding POCT activities and issues. The POCT coordinator supported the pathologists in developing policies and procedures, designing and interpreting method validations, and in communicating with clinicians. Periodically, the POCT coordinator inspected POCT sites to ensure that proper procedures are being followed and documentations for patient identification, patient preparation, specimen collection, specimen ID, specimen preservation and processing, and result reporting are in place. It was couple of months later that our POCT coordinator assistant was also hired and trained. Therefore depending on the size and nature of the POCT service, a POCT coordinator assistant may also be required.

Responsibilities of a POCT coordinator were clearly outlined in four domains of quality assurance, corrective action, training and education, and administration. To ensure quality assurance it was the responsibility of POCT coordinator to check if all POCT systems are in compliance with accreditation needs, to provide evaluation of the analytical performance and method validation of POCT devices, to monitor performance of internal QC and take corrective actions, to schedule proficiency testing surveys, to monitor performance of proficiency testing at each POCT site and take corrective actions, to monitor POCT quality indicators and share with quality improvement coordinators committee meetings and to conduct POCT site internal audits and take regular feedbacks from POCT site supervisors. All technical problems and complaints were to be handled by the POCT coordinator. He was also responsible to arrange for POCT user training, competence assessment and maintaining POCT users' records. He was the person responsible for coordinating POCT activities and connecting with other health care professionals. He acted as the liaison between nursing and laboratory personnel. Selection and installation of POCT instrument, maintaining the in house inventory of POCT tests, review and update of POCT standard operating procedures and policy manual were all his duties. He also ensured that updated POCT manual was available at all POCT sites and implemented safety rules and regulations at all POCT sites. Checking maintenance schedules and coordinating between Interdisciplinary and End user Committees were also done by him.

The Aga Khan University Hospital Clinical Laboratory has sections of clinical chemistry, clinical microbiology, haematology, histopathology, molecular pathology, blood bank and transfusion services. The POCT program came under the domain and responsibility of clinical chemistry. A middleware Cobas IT 1000 was installed to connect the Clinical Chemistry's laboratory information system (LIS) with hospital information system (HIS). POCT coordinator controlled all POCT activities via Cobas IT 1000.

This included review of POCT end users details and competence assessment, POCT patient results, QC transmission and validation. IT 1000 has the ability to communicate bi-directionally with HIS and POCT devices. In order to control POCT operation and the quality of patient care, and to ensure that results are integrated into and being networked with the LIS, the establishment of new relationships among the laboratory, clinicians and the Information Technology (IT) was needed. For establishing goals, addressing compliance matters and setting future directions of the program two committees were established; an interdisciplinary committee and end user committee. The interdisciplinary committee had representation from various departments and was headed by the laboratory director and pathologists while end user committee was headed by the POCT coordinator and had pathologists and nursing managers from various departments as members.

Responsibilities of interdisciplinary committee were as follows:

- To establish/allocate POCT system in defined areas.
- Any proposal to establish POCT must be referred to this Committee for approval via the POCT coordinator.
- To evaluate & select equipment for testing.
- To select appropriate methodology.
- To assign responsibility for test performance.
- To define policy/procedure for record keeping /documentation.
- To assess POCT impact or outcome.
- To assess whether POCT meets safety and quality standards.
- To ensure POCT meets the requirements in relation to protecting data, patient confidentiality and risk management.

Responsibilities of end user committee were as follows:

- To discuss ongoing issues and problems with POCT program.
- Recommendations for change should be forwarded to the interdisciplinary committee for consideration.
- To discuss various compliance reports.

Identifying POCT site supervisors

Nurse Directors or nursing managers qualify as POCT site supervisors. They are responsible for day to day supervision and oversight of POCT users performing and reporting test results in their respective areas or POCT sites. The responsibilities of POCT site supervisors were well defined in the POCT program manual before implementation at our institute. They were made responsible to set up and maintain a system of regular internal QC checks, maintain equipment in a manner appropriate to the proper collection, handling, preparation, testing and storage of specimens and operation of test results and patient's reports.

POCT site supervisors reviewed QC data on weekly basis to assure that testing and corrective action is taken and documented. They would contact laboratory and cooperate with troubleshooting and corrective measures if performance seemed unsatisfactory. They were the ones who would identify POCT user for competency assessment and ensure competency assessment of every POCT user. They documented certification and competency assessment of every POCT user in their respective sites. All requests for new POCT systems were made through them via the POCT committee in accordance with the selection and procurement criteria. POCT site supervisors also maintained in-house inventory, place orders for the required reagents and consumables from the hospital logistics and were responsible for

administration of the daily operation of POCT at their respective site.

Selection of POCT tests and analyzers

Comparison of the available equipment from different vendors according to the preset criteria and specific standards was done by the POCT team members from Pathology, Biomedical Engineering (BE), and Material and Management Division (MMD) (16-18). The test menu proposed included arterial blood gases, electrolytes, glucose and urine dipstick analysis. The team realized that the POCT instruments or devices must be user friendly, robust both in terms of storage and usage, capable of producing results consistent with the medical needs, less costly and safe.

The system performance of the proposed POCT devices was evaluated. Power and network requirements were considered and POCT site visits were conducted before making any decision.

Regulations mandate documentation of method selection, method validation before patient testing is performed for each POCT device placed. Validation of equipment, test method verification and instrument to instrument comparison was done by our POCT team according to CLSI guidelines at the Central Laboratory by POCT coordinator (19).

Protocol for POCT method validation according to CAP and CLIA standards included accuracy, precision, verification of reportable range and analytical measuring range, POCT inter-instrument comparison and comparison with bench top analyzers placed in the central laboratory. Reagent shipments and lot numbers were validated and tracked.

Management of consumables and reagents was and still is procured in a cost-effective manner to the clinical unit concerned. POCT costing was done which included the fixed capital cost (instrument, proficiency testing survey cost, service contract for vendor, ancillary infrastructure etc.) and variable cost (reagent consumption, internal controls, consumables etc.).

Data capture and connectivity

Improvements in testing technologies and the advancements in specialized informatics for POCT have greatly improved the ability of hospitals to manage their POCT program (15) (14, 20).

The benefits of POCT are enhanced when results and records are directly downloaded into a laboratory information system (14, 21). For accreditation and patient safety, trail must link each patient result to the operator to his/ her training records, the reagent lot used for testing and its validation, and the POCT device to its validation and maintenance. Managing the quality of large volumes of POCT data was a continuing challenge for our POCT team.

Confidentiality, security, legality, compatibility, interoperability, timeliness, and convenience of processes, records, communications, and software were reviewed by the IT support team of our institute. Connectivity of POCT equipment to a middleware POCT data management system (server) and then to integrated laboratory management system was established. The connectivity in place was bidirectional, from LIS to middle ware and then back to LIS. Through IT 1000 (middle ware) specific configuration were assigned to all POCT devices for internal quality control.

A QC lock provision was set up in all devices and at 8 AM all meters automatically are on QC lock. This does not allow patient testing until two level of quality control are passed. The middleware is also used to assign new lot configuration of reagents and control. The ‘client BG link’ connected with IT1000 gives facility to control all ABG analyzers remotely from the central laboratory.

All onsite POCT instruments and POCT users were connected via this server to the LIS. To reduce medical errors barcode was introduced for all POC tests. The POCT instruments, regardless of their site location, generated data related to sample analyses (POCT user and patient identification and test results), quality control and the instrument itself (calibration and maintenance). These voluminous data were managed through a middleware further linked to LIMS.

Table 4 Components of POCT training curriculum of POCT users

List of policies, processes and modules in the POCT training curriculum
Entry qualifications of POCT users.
Sample requirement, sample collection and handling including any special requirements.
Positive patient and operator identification before testing.
Stability of sample and reagents.
Device theory of operations and steps in analyte measurement.
Timely routing of results to the decision maker and the appropriate operator response to results that are outside predefined limits.
Clinical significance of results.
Actions to be taken in case of critical or unusual results.
Sources of common errors.
Maintenance, calibration and cleaning of instruments.
Performance of QC and review of POCT safety and security policies.
Information systems that support POCT, the rationale for using them, the benefits they provide, and the problems inherent in their implementation and use.
Safe disposal of the sample and sampling device.
POCT device error codes, their meanings and what to do if the device generates an error.
Documentation of all records and reports.
Maintenance tasks and consumable storage.
Who to call if there is a problem with the device or stock needs replenishing.

With the help of LIMS and hospital integrated management system a mechanism for record keeping, archiving, billing, and data entry into the electronic medical record was ensured.

Staff training and competency assessment

Unlike the modern central laboratory where the bulk of testing is conducted on few analyzers by a core group of skilled technologists, POCT is conducted by a variety of clinical staff on multiple devices in many locations (11, 22). Training and competency assessment of all POCT users was a logistical challenge. Like all other organization attrition was a constant challenge faced by the 'POCT Program implementation team'. (23). The POCT training plan and curriculum were developed in line with the CLIA'88 and CAP standards by the chemical pathologists and shared with Nursing Education Service (NES) for implementation as nursing staff are typically the largest percentage of POCT operators . CLIA'88 requirements for competency assessment involve "evaluating the competency of all testing personnel and assuring that staff maintains their competency to perform test procedures and report test results promptly, accurately and proficiently". CLIA'88 requires that laboratories have on-going mechanisms to monitor accurate patient test management. Competency assessment is one method used to ensure those POCT users that perform POCT are proficient in test procedure and reporting test result. The POCT training program at our institute ensured that testing personnel met regulatory requirements and provided regularly scheduled review of training and techniques. The POCT personnel training program chiefly consisted of three components: initial formal POCT training, POCT recertification and POCT competency assessment. Training of trainers (TOT) and assessing their competence to provide training to others was done. Training was performed of TOTs from NES followed by training of the end users. Fifty-eight trainers were trained from NES with the support from POCT Coordinator and manufacturer. The TOTs further conducted more than 100 sessions (3-4 lectures/ward) to train more than 1000 nursing staff. Training included classroom training, hands on training of POCT device correct operation and assessment. Training curriculum included all phases of the testing process (Table 4).

Competency assessment included observation of technique, written examination, analysis of quality control or specimens with known values, demonstration of maintenance, recording of test results, and evaluation of communication and problem-solving skills. Competency was documented through certifications that assured that individual POCT operators met training and experience standards. After POCT certification, the POCT users were issued barcoded access to use of the POCT devices. Recertification of this 'competency certificate' is done annually or sooner if need arises. Furthermore, all training and ongoing competency verification records were and are still maintained via the online connectivity server by the POCT coordinator. POCT training program ensures that testing personnel meet regulatory requirements and provides regularly scheduled review of training and techniques (24).

QUALITY ASSURANCE

In principle, no difference between POCT and conventional laboratory testing exists with respect to pre- and post-analytical errors (25). Therefore, the entire diagnostic process must be considered in quality assurance (26). Clinicians and nursing staff may fail to comprehend the importance of quality control and correct documentation when performing POCT (27). While ensuring the quality of POCT compliance with regulatory guidelines it is mandatory for institutions to maintain their accreditation status (28,

29). The analytical goals for POCT were equivalent to those used for our central laboratory and it was ensured that the use of POCT does not compromise standard of patient care and clinical decision making (28-30). Both internal quality control (IQC) and external proficiency testing (PT) programs were an essential component of QA for POCT program at AKUH (31).

Three main IQC requirements were addressed: procedure established for IQC at appropriate frequency, QC material procurement and correction of nonconformities. To assure that the POCT devices were working correctly all POCT users were trained for running and monitoring IQC along with remedial actions before patient testing. It should also be noted all the POCT devices had advanced levels of connectivity and the ability to electronically capture and transmit results to the middleware. The POCT management middleware offered features such as operator and patient ID lockout, QC lockout, remote configuration and management of consumables, improving efficiency and giving us strict control of our testing program. With the help of IQC the reproducibility or precision was monitored by the central laboratory on routine basis.

Policy for proficiency testing (PT) was outlined as part of POCT program. Before POCT execution all PT surveys were identified from CAP and acquired. Comparison of results and performance across different POCT sites was done by the central laboratory and communicated to all site supervisors routinely. Sub-optimal performance in PT and internal quality control was brought to the immediate attention of the POCT committees, which then determined corrective action. The POCT coordinator ensured that PT surveys generate accurate results, regardless of the location. Control of training and competence assessment, policies, procedures and IQC and PT are now under the guidance/oversight of clinical laboratory. The connectivity for our POCT program and the data management capabilities has given us the ability to monitor our whole program of >1500 operators and to produce accurate audit trail. Our POCT operators understand that the laboratory is overseeing every aspect of testing and monitoring it closely along with regular onsite inspections.

SERVICE EXECUTION

Pilot project and expansion

Finally, a POCT execution plan was laid down. A live demonstration on connectivity was performed in the laboratory before making it live at the patient testing sites. POCT reporting format was finalized and essential components were made part of POCT report (Table 5). Training refresher for all POCT users, review of instruments installation and inventory check was performed by POCT coordinator. A 24/7 hotline was in place to resolve POCT related query and complaints. A POCT contingency plan was put forward and the POCT team was open to suggestions or complaints based on the feedback from POCT users and physicians. The POCT team conscientiously monitored the whole process for one week at each site and implementation was signed off gradually one after another. Fifty-nine glucometers, five urine analysis devices and five arterial blood gas analyzer were installed at 22 sites (including emergency department, critical care units and wards). The initial week or the transition phase was the toughest time once POCT implementation was introduced at these various sites. The issues faced were frequent QC failures because of incorrect QC identification, frequent comparison of POCT results to central laboratory testing, decreased utilization of POCT by end users, frequent training refreshers and instrument breakdown because of mishandling. Laminated posters or flyers with simple step-by-step instructions on how to perform a test on a patient and how to conduct quality

Table 5 Elements of POCT report

Essential features to be specified in POCT reports
Patient identification and patient location
Demographics of patient
Time and date of test when performed
Patient results with units
Type of sample
Distinction that the test was done on POCT device and not in central laboratory.
Identification of POCT operator who performed the test
Reference interval of analyte
Treating physician's identification
The identification of the laboratory that issued the report

management (QC and PT) testing procedures into a practical, workable format was distributed at all POCT sites as a training refresher.

Clinical audit and ongoing POCT compliance

All POCT programs need to be observed and evaluated periodically in order to assure that the program is meeting the needs of patients, testing personnel and hospital (22). Once our POCT program was in place, a clinical audit was conducted by the institute's 'Quality Assurance Department'.

All POCT sites were audited and assessed for the policy, procedure and protocols, POCT users' knowledge, skills and practices. Most of the POCT users were aware about the procedures and policies, daily QC checks and reporting of the equipment related complaints. Daily QC checks were maintained and all POCT users were knowledgeable about the disinfection protocols. However audit revealed underutilization of few POCT instruments, manuals not easily accessible to end users and inadequate knowledge of some POCT users regarding result reporting and corrective action to be taken.

Based on audit findings and POCT team discussions some quality performance indicators were introduced in the practice to monitor POCT on a continuous basis. These include patient to QC testing ratio, moving average of blood glucose in the hospital and PT survey monitoring. The laboratory POCT team prepared for CAP inspection two years later and got accredited by CAP in 2016 and again in 2019.

The POCT program is now under strict oversight of CAP. Supervision of a POCT program requires continuous attention to POCT instruments and users management, competency management,

review of IQC and PT, presenting IQC and PT results in QA meeting of the institute, data monitoring, inventory management, monitoring all POCT devices and their remote access, processes of introducing new POCT in the institute and day to day issues (32).

CONCLUSION AND WAY FORWARD

The POCT allows rapid diagnostic and screening test results. Concerns over the quality of results and difficulties in managing the documentation have created challenges to the extensive adoption of POCT in hospitals in the developing world. A clearly defined organizational structure should be put in place for proper functioning and optimum utilization of each POCT unit. With our experience of implementing POCT program the key to success of establishment of POCT infrastructure was a dedicated project lead and team work. POCT implementation requires multidisciplinary, multimodal approach involving all stakeholders, giving respect to each other and with effective communication. In spite of major improvements in technology, assuring the quality of POCT remains challenging. This review may guide and assist other healthcare providers in implementing POCT effectively for improving patient safety and outcome.

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Prevalence of liver function test abnormality and associated factors in type 2 diabetes mellitus: a comparative cross-sectional study

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liver function tests, diabetes mellitus, liver disease

ABSTRACT

Background:

Determining liver biomarkers can help to screen and facilitate early management of potential liver diseases. However, such studies are scarce in the present study area. Therefore, our study planned to assess the prevalence of liver function test abnormality and associated factors among Type 2 Diabetes Mellitus (T2DM) patients.

Methods:

A comparative cross-sectional study was conducted at the University of Gondar Comprehensive Specialized Hospital from January 1, 2018 to May 20, 2018 among 159 T2DM patients and 159 nondiabetic controls. Clinical, lifestyle, anthropometric data and 5 ml of blood were collected from all study subjects. Liver function tests (LFTs), lipid profiles and fasting blood sugar were determined. Systematic random sampling technique was used to select the study subjects. Binary logistic regression and bivariate correlation was used to assess association of factors with outcomes and p value of ≤0.05 was considered as significant.

Results:

Overall, 53 (33.3%) of T2DM had one or more liver test abnormality above the upper limit of the normal (ULN) reference range. Alanine aminotransferase was the most frequently raised liver enzyme in T2DM (n=37, 23.3%). The mean value of LFTs was significantly different between T2DM and the control group. Alcohol drink, sex and age were found to be a significant factor for impairment of LFTs.

Conclusion:

The prevalence of abnormal LFTs was higher in T2DM patients than nondiabetic control group. Hence, we recommended the utilization of LFTs to monitor liver conditions in T2DM patients.

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INTRODUCTION

Diabetes mellitus(DM) is a group of metabolic disorders of carbohydrates, lipids and proteins characterized by hyperglycemia (1). Globally, more than 415 million people, aged 20-79 years, were affected by DM and the figure is expected to rise up to 642 million in 2040. An epidemic growth of DM has occurred in developing countries in which 75% of patients with DM live in the low and middle-income countries. In addition, DM affects the working age in the low and middle-income countries (2).

The exact pathophysiological mechanism of DM to induce abnormalities in liver biomarkers is still unclear. The first possible explanation that DM induces liver function abnormality is the deposition of fat in the liver which is the characteristics of nonalcoholic fatty liver disease (NAFLD). The other possible assumption is the vulnerability of individuals with metabolic syndrome like DM to inflammation of the liver which alters the function of liver and induce a change in liver biomarkers (3).

Liver function tests (LFTs) are used in clinical practice to screen liver disease, to monitor the progression of a known liver disease and to monitor the effects of potentially hepatotoxic drugs. The most commonly used LFTs include the serum aminotransferases, alkaline phosphatase (ALP), bilirubin, total protein (TP), albumin, and prothrombin time. Measurement of serum aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serve as a marker of hepatocytes injury. ALP, gamma-glutamyltranspeptidase (GGT) and bilirubin act as markers of biliary function and cholestasis whereas TP, albumin and prothrombin time reflect liver synthetic function (4).

The prevalence of abnormal LFTs among DM patients is still controversial (4). In addition, there is limited evidence about the relationship between abnormality in LFTs and DM patients in the study area. Awareness of DM as a significant risk factor for liver injury may help for early diagnosis and interventions and derive health-promoting policies which encourage action to prevent liver diseases in the future. Therefore, the objective of this study was to assess LFTs abnormality and associated factors among patients with T2DM attended at the University of Gondar Comprehensive Specialized Hospital.

MATERIALS AND METHODS

Study design and subjects

A facility based comparative cross sectional study was conducted at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia, from January 1 to May 20, 2018. The study included a total of 318 participants; 159 T2DM patients and 159 non-diabetic controls. A systematic random sampling was used to select study participants. We included adult (≥18 years) T2DM and nondiabetic controls. Patients with history of liver disease, pregnant women and patients on drugs known to have effect

on liver function (except antidiabetic therapy) were excluded from the study.

Data collection and laboratory methods

Socio-demographic characteristics and clinical data were collected by trained nurses using a pretested semi-structured questionnaire. Trained laboratory technologists collected five milliliter (5 ml) of blood sample. Then after centrifuging the clotted sample, the serum was analyzed for LFTs, lipid profiles and FBS by using Mindray BS-200E chemistry analyzer (Shenzhen Mindray Bio-Medical electronics Co. Ltd, China). The remaining serum was also used for HBsAg and HCV anti-body detection by using One Step Cassette Style HBsAg Rapid Test and EUGENE® anti-HCV rapid test, respectively. The quality of each test was maintained by strictly following the standard operating procedures. Quality control was run daily prior to each test. Completion, accuracy and clarity of the collected data was checked regularly.

The interpretation of test results was based on the reference range recommended by the manufacturers' instructions. Serum level of alkaline phosphatase (ALP) >306U/L, total bilirubin >1.2mg/dl, direct bilirubin>0.2mg/dl, total protein (TP) <6.6g/L and albumin <3.5g/L was considered as abnormal. The level of ALT >32U/L and AST >31U/L for female and ALT >42U/L and AST >37U/L for male was classified as abnormal. Fasting blood sugar (FBS) >115mg/dl, triglyceride (TG) >200mg/dl, total cholesterol (TC) >190mg/dl, HDL-cholesterol (HDL-c) <40mg/dl and LDL-cholesterol (LDL-c) >100mg/dl were considered as abnormal.

Physical activity is defined as if a study participant was doing sport by allocating regular time. Sweet eating can be defined as an eating behavior in which study participants consumed carbohydrates enriched foods such as cookies and chocolate regularly.

Anthropometric measurement (weight, height, waist circumference) was measured according to WHO guideline by trained nurses. Body mass index (BMI) was calculated as weight divided by height squared (kg/ m^2) and classified as underweight (BMI <18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (BMI=25-29.9 kg/m^2) and obese (BMI ≥ 30kg/m^2)(5). Waist circumference (WC) >88 centimeters for female and WC>101 centimeters for male was taken as high(central obesity)(5). Blood pressure was taken by qualified personnel using an analogue sphygmomanometer and stethoscope. Systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or current use of blood pressure-lowering medication was used to define hypertension (6).

Data analysis and interpretation

After cleaning and coding, data was entered and analyzed with SPSS version 20 statistical package. Data is presented as mean ± standard deviation (SD) or a percentage (%). Descriptive statistics of frequency distributions, summary and variability measurements are used. Binary logistic regression and bivariate correlation were used to determine the relationship between dependent and independent variables. Factors with p-value ≤0.25 had been transferred to multiple binary logistic regression analysis. A p-value ≤0.05 was considered as statistically significant.

Ethical consideration

Ethical clearance was obtained from the School of Biomedical and Laboratory Sciences Research and Ethics committee. Written informed consent was obtained from study participants before the commencement of data collection. There was no financial compensation or provision for the study participants. To ensure confidentiality of data, study participants were identified using codes and unauthorized persons had no access to the collected data.

RESULTS

Characteristics of study subjects

The mean ± SD age of a control group was 52±13.22 years, ranging between 24 and 80 years and the mean ± SD age of T2DM was 55±11.025 years, ranging from 32 to 85 years(p=0.23). Overall, 82(51.6%) of T2DM patients and 76(47.8%) of a control group were male and 87(54.7%) of T2DM and 87(54.7%) of a control group were under the age of 55 (Table 1).

Prevalence of abnormal liver function tests

Elevated ALT was found in 37 (23.3%) of T2DM and 4 (2.5%) of a control group. Elevated AST was observed from 34 (21.4%) of T2DM and 3 (1.9%) of a control group. On the other hand, 19 (11.9%) of T2DM patients and 1 (0.6%) of controls had decreased level of TP respectively. One or more test abnormality was observed in 53 (33.3%) of T2DM patients and 6 (3.8%) of controls whereas 30 (18.9%) of T2DM patients and 3 (19%) of controls revealed an abnormal level of LFTs in two or more tests.

DISCUSSION

Mean values of ALP, ALT, AST, total and direct bilirubin were significantly higher in T2DM than the control group (P for trend ≤0.04). On the other hand, the mean values of albumin and TP were

Table 1 Sociodemographic, clinical and anthropometric characteristics of type 2 diabetes mellitus patients and nondiabetic controls, Gondar, Northwest Ethiopia, 2018

Variables		Study subjects		Total N (%)
		T2DM patients N (%)	Control group N (%)	
Sex	Male	82(51.6)	76(47.8)	158(49.7)
	Female	77(48.4)	83(52.2)	160(50.3)
Age	<55	87(54.7)	87(54.7)	174(54.7)
	≥55	72(45.3)	72(45.3)	144(45.3)
Alcohol drink	Yes	56(35.2)	65(40.7)	121(38.1)
	No	103(64.8)	94(59.3)	191(61.9)
Coffee drink	Yes	112(70.4)	115(72.3)	227(71.4)
	No	47(29.6)	44(27.7)	91(28.6)
Physical exercise	Yes	40(25.2)	47(29.6)	87(27.4)
	No	119(74.8)	112(70.4)	231(72.6)

Body mass index	Underweight	9(5.6)	4(2.5)	13(4.1)
	Normal weight	77(48.4)	154(96.9)	231(72.6)
	Over weight	47(29.6)	1(0.6)	48(15.1)
	Obese	26(16.4)	-	26(8.2)
Waist circumference	Normal	124(78)	157(98.7)	281(88.4)
	Central obesity	35(22)	2(1.3)	37(11.6)
Duration diabetes mellitus	0-5	99(62.3)	-	-
	6-10	31(19.5)	-	-
	>10	29(18.2)	-	-
Systolic blood pressure	Normal	122(76.7)	158(99.4)	280(88.1)
	High	37(23.3)	1(0.6)	38(11.9)
Diastolic blood pressure	Normal	142(89.3)	149(93.7)	291(91.5)
	High	17(10.7)	10(6.3)	27(8.5)
Hypertension	Present	67(42.1)	11(6.9)	78(24.5)
	Absent	92(57.9)	148(93.1)	240(75.5)
Family history of diabetes mellitus	Yes	19(11.9)	12(7.5)	31(9.7)
	No	140(88.1)	147(92.5)	287(90.3)

Note: N=number of participants; T2DM=type 2 diabetes mellitus; %=percentage.

significantly lower in T2DM than controls. A similar finding regarding ALT and AST (7-9) and TP and albumin (8) was reported.

The prevalence of abnormal LFTs was higher than the prevalence observed in control group. The most frequent abnormal LFT was ALT (23.3%, 95%CI=17%-30.2%) which was followed by AST (21.4%, 95%CI=14.5%-28.3%). This is in line with the studies conducted in Finland (10), Scotland (11) and England (12) reported a 17%, 23.1% and 25.6% prevalence of abnormal ALT in T2DM patients, respectively. The prevalence of abnormal ALP was 10.7% (6.3%-15.7%) which is comparable with 8.9%, reported from Algeria (13). The prevalence of elevated ALT and AST was higher than the prevalence reported from Algeria (13)

Table 2 Comparison of clinical and biochemical parameters between T2DM patients and nondiabetic controls, Gondar, Northwest Ethiopia, 2018

Parameters	Mean ± Standard deviation(SD)		P value	Units of measure-ment
	T2DM patients (n=159)	Controls group (n=159)		
Fasting blood sugar	182.60±74.375	84.25±51.56	≤0.001	mg/dl
Triglyceride	188.45±103.62	86.58±58.84	≤0.001	
Total cholesterol	191.05±77.07	139.18±35.44	≤0.001	
High density lipoprotein cholesterol	44.18±11.940	52.28± 8.156	≤0.001	
Low density lipoprotein cholesterol	105.33±56.0	55.55±8.53	≤0.001	
Systolic blood pressure	135.52±16.52	118.72 ± 7.81	≤0.001	mmHg
Diastolic blood pressure	81.30±9.02	79.63 ± 5.2	0.04	
Waist circumference	94.00±8.81	78.14±8.26	≤0.001	centimeter
Body mass index	26.74±18.79	23.30 ± 2.53	0.02	kg/m^2
Alkaline phosphatase	165.55±81.610	118.38±23.628	≤0.001	U/L
Alanine aminotransferase	37.57±35.831	17.89±7.097	≤0.001	
Aspartate aminotransferase	35.2±32.278	17.89±7.067	≤0.001	
Total protein	7.469±.8461	8.005±.7583	0.003	g/dl
Albumin	4.602±.6736	4.930±.3262	0.004	
Total bilirubin	.553±.3907	.418±.2449	0.006	mg/dl
Direct bilirubin	.169±.1680	.140±.0575	0.04	

Note: g/dl=gram/deciliter; Kg/m2=kilogram/square meter; mg/dl=milligram/deciliter; mmHg=millimeter mercury; U/L=unit/liter.

Figure 1 **Number of study subjects with liver function tests abnormality in type 2 diabetes mellitus patients and controls, Gondar, Northwest Ethiopia, 2018**

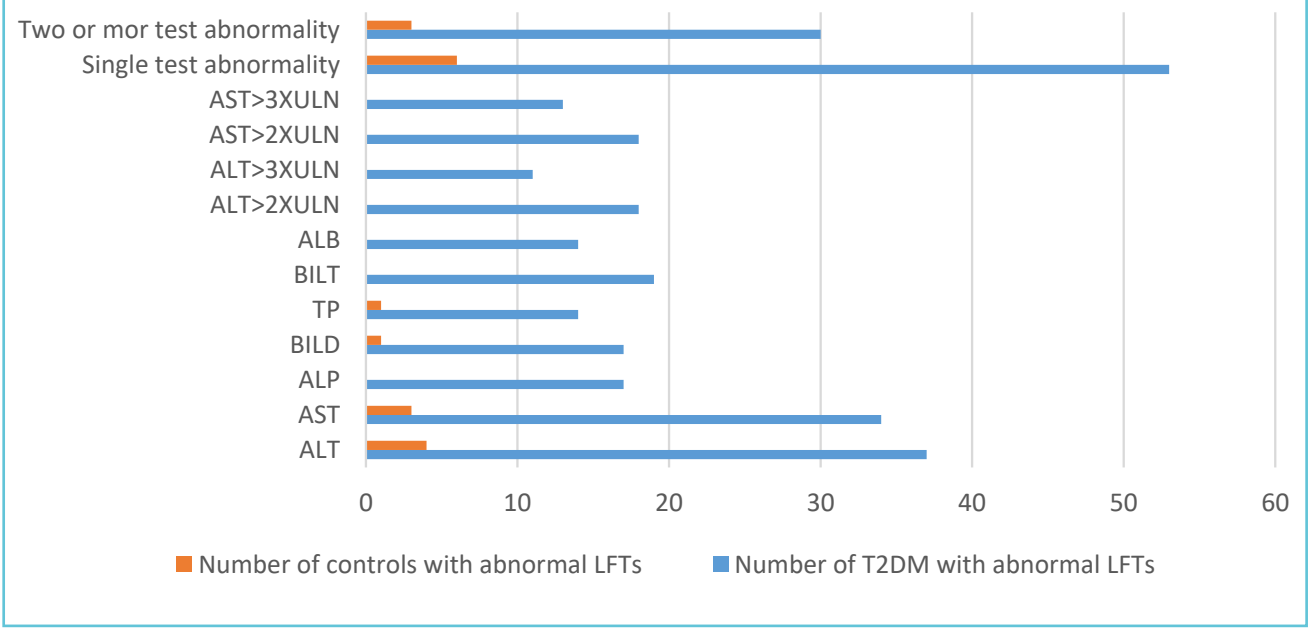

Figure 2 **Percentage distribution of liver function tests abnormality in type 2 diabetes patients and controls, Gondar, Northwest Ethiopia, 2018**

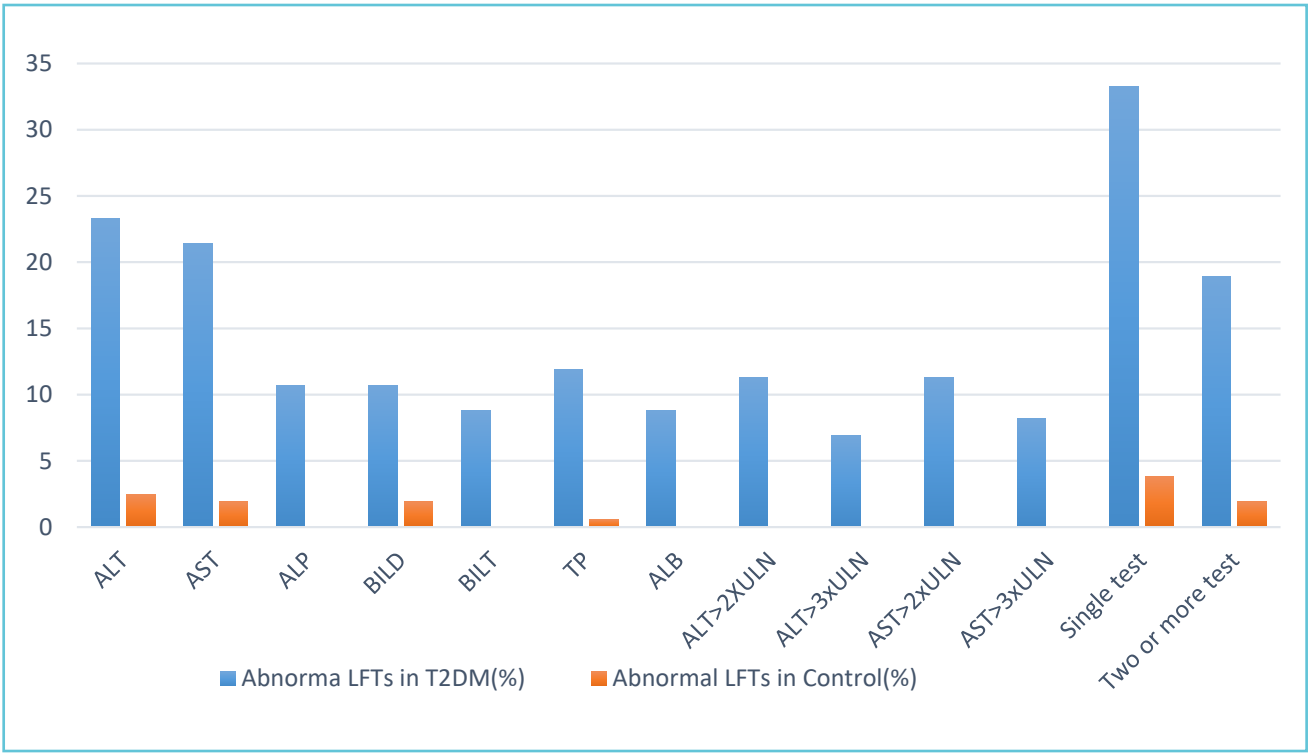

Table 3 Bivariate correlations among continuous variables in T2DM, Gondar, Northwest Ethiopia, 2018

		DM duration	Systolic BP	Diastolic BP	WC	BMI	TG	FBS	TC	HDL-c	LDL-c
ALP	r	-0.245	0.289	0.273	0.430	0.329	0.728	0.772	0.812	-0.628	0.792
	P value	0.002	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
AST	r	-0.200	0.283	0.289	0.443	0.327	0.731	0.786	0.847	-0.680	0.849
	P value	0.011	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
ALT	r	-0.198	0.315	0.305	0.450	0.395	0.737	0.797	0.840	-0.686	0.855
	P value	0.012	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
TP	r	0.173	-0.233	-0.203	-0.417	-0.241	-0.609	-0.692	-0.773	0.592	-0.695
	P value	0.029	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
ALB	r	0.119	-0.261	-0.211	-0.424	-0.305	-0.671	-0.759	-0.822	0.694	-0.784
	P value	0.134	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
BILT	r	-0.191	0.150	0.206	0.303	0.241	0.658	0.668	0.815	-0.584	0.764
	P value	0.016	0.007	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
BILD	r	-0.134	0.162	0.236	0.254	0.274	0.528	0.554	0.690	-0.525	0.690
	P value	0.092	0.004	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001

Note: 'r' denoted correlation coefficient; ALB=albumin; ALP=alkaline phosphatase; ALT=alanine aminotransferase; AST=aspartate aminotransferase; BILD=direct bilirubin; BILT=total bilirubin; BMI=body mass index; BP=blood pressure; FBS= fasting blood sugar; HDL-c=high density lipoprotein cholesterol; LDL-c=low density lipoprotein cholesterol; TC=total cholesterol; TG=triglyceride; TP= total protein; WC=waist circumference.

Table 4 Binary logistic regression analysis of associated factors with different abnormal LFTs in patients with type 2 diabetes mellitus, Gondar, Northwest, Ethiopia, 2018

Factors		Elevated ALP	Elevated AST	Elevated ALT	Decreased TP	Decreased ALB	Elevated BILT	Elevated BILD
Male	COR (95%CI)	8.4 (4.2-11.7)	3.3 (1.4-6.8)	3.9 (1.6-7.8)	2.8 (1.1-7.2)	6.4 (0.9-8.6)	6.4 (2.9-8.1)	3.4 (0.9-6.3)
	P value	0.038	0.005	0.002	0.03	0.08	0.038	0.068
	AOR (95%CI)	0.6 (0.2-2.4)	2.1 (1.2-3.8)	1.2 (1.01-3.4)	1.1 (0.4-3.3)	1.2 0.4-3.6)	1.0 (0.3-3.3)	0.9 (0.3-2.7)
	P value	0.50	0.014	0.047	0.80	0.80	1.0	0.80
Age<55	COR (95%CI)	0.4 (0.2-1.1)	0.6 (0.3-1.3)	0.4 (0.2-0.9)	0.5 (0.2-1.2)	0.4 (0.1-1.05)	0.3 (0.1-0.8)	0.5 (0.2-1.2)
	P value	0.068	0.22	0.033	0.11	0.06	0.024	0.11
	AOR (95%CI)	0.4 (0.1-1.1)	0.2 (0.1-0.8)	0.3 (0.1-0.9)	0.3 (0.1-0.8)	0.5 (0.2-1.3)	0.3 (0.1-1.02)	0.5 (0.2-1.4)
	P value	0.054	0.041	0.035	0.012	0.13	0.055	0.22
Butter	COR (95%CI)	1.9 (1.1-3.1)	2.1 (1.3-3.4)	2.1 (1.3-3.4)	1.5 (0.9-2.5)	-	1.9 (1.1-3.1)	1.9 (1.2-3.2)
	P value	0.019	0.003	0.003	0.15	-	0.019	0.012
	AOR (95%CI)	1.7 (0.9-3.4)	2.1 (1.2-3.4)	1.4 (1.01-2.6)	1.2 (0.7-2.3)	-	1.5 (0.8-2.8)	1.6 (0.9-3.0)
	P value	0.13	0.014	0.045	0.5	-	0.22	0.12

Alcohol	COR (95%CI)	11.7 (3.7-16.9)	8.4 (3.7-18.9)	11.2 (4.7-17.2)	6.5 (2.5-16.8)	4.1 (1.5-11.1)	6.5 (2.4-12.3)	7.0 (2.6-15.2)
	P value	0≤0.001	≤0.001	≤0.001	≤0.001	0.005	≤0.001	0≤0.001
	AOR (95%CI)	5.9 (3.4-8.6)	6.4 (2.6-11.2)	6.0 (1.8-12.1)	6.1 (2.1-12.3)	4.2 (1.4-8.9)	5.1 (1.6-11.6)	6.0 (2.0-10.9)
	P value	≤0.001	.001	≤0.001	.001	.011	.006	0.002
Coffee	COR (95%CI)	0.3 (0.1-0.8)	0.7 (0.3-1.2)	0.5 (0.3-1.1)	0.4 (0.2-1.0)	0.3 (0.1-0.8)	0.5 (0.2-1.2)	0.5 (0.2-1.3)
	P value	0.015	0.14	0.10	0.056	0.019	0.13	0.18
	AOR (95%CI)	0.2 (0.1-0.7)	0.4 (0.2-0.9)	0.4 (0.2-1.2)	0.4 (0.1-0.9)	0.3 (0.1-0.9)	0.1 (0.02-0.9)	0.5 (0..2-1.4)
	P value	0.01	0.047	0.09	0.045	0.028	0.043	0.16
Sweet drink	COR (95%CI)	5.9 (2.3-15.3)	3.4 (1.5-7.8)	5.1 (2.2-11.9)	2.9 (1.1-7.3)	-	4.6 (1.8-12.0)	3.3 (1.3-8.6)
	P value	0≤0.001	0.004	0≤0.001	0.028	-	0.002	0.013
	AOR (95%CI)	5.1 (1.7-11.5)	2.2 (0.7-6.9)	6.2 (1.7-16.0)	2.6 (1.03-9.9)	-	3.0 (0.8-11.5)	2.4 (0.6-8.7)
	P value	0.008	0.19	0.006	0.048	-	0.10	0.20
T2DM	COR (95%CI)	-	14.7 (4.4-18.8)	11.8 (4.1-16.9)	21.4 (2.8-42.2)	-	-	6.2 (1.8-11.7)
	P value	-	0.001	0.001	0.003	-	-	0.004
	AOR (95%CI)	-	4.2 (1.6-10.6)	3.6 (1.8-7.1)	5.2 (1.2-8.8)	-		2.5 (1.2-5.2)
	P value	-	0.003	0.001	0.013	-	-	0.012

Note: '-' denotes not applicable; ALB=albumin; ALP=alkaline phosphatase; ALT=alanine aminotransferase; AOR=adjusted odds ratio; AST=aspartate aminotransferase; BILD=direct bilirubin; BILT=total bilirubin; CI=confidence interval; COR=crude odds ratio; TP=total protein; T2DM=type 2 diabetes mellitus.

which observed 13.9% for ALT and 10% for AST. The difference might be attributable to the difference in medical care, living standard and knowledge of the patients on the risk factors.

It was noted that 33.3% (95%CI=26.4%-41%) of T2DM had one or more test abnormality. This finding is consistent with the previous study conducted in Scotland (11) which reported 29.1%. Disagreed with our result, studies from South Africa (14) and Finland (10) reported a 46% and 57% prevalence of one or more test abnormality. The possible explanation of the difference in prevalence might be due to the utilization of different cutoff values, which are influenced by sociodemographic characteristics such as sex, age and ethnicity (15). The prevalence of two or more test abnormality was 18.9% (95%CI=12.6%=25.8%). This is similar with a finding from Sudan (8) reported 24%, but lower than the prevalence (27%) reported from Finland (10). Among T2DM patients, 11.3% (6.9%-16.4%) revealed elevated ALT and AST each beyond two times the ULN whereas 6.9% (3.1%-11.3%) and 8.2% (3.8%-12.6%) of T2DM was found with an elevated level of ALT and AST respectively beyond three times the ULN. In line with our finding, a retrospective study from South Africa (14) showed a 9% and 6% prevalence of abnormal liver enzymes over two times ULN and three times ULN in T2DM respectively.

It was noted that WC, BMI, TC, TG, LDL-c and FBS were significantly positively correlated with elevated level of ALP, ALT, AST, total bilirubin and direct bilirubin (P≤0.001). But, these were significantly negatively correlated with TP and albumin (P≤0.001). HDL-c was significantly negatively correlated with liver enzymes and bilirubin(P≤0.001) while it was significantly positively correlated with the level of TP and albumin (P≤0.001). A study from South Africa (14) has previously reported that patients with abnormal liver enzyme were significantly associated with dyslipidemia. Another study from China (16) demonstrated a significant positive correlation between elevated ALT and WC, BMI, TC, TG, LDL-c and FBS. In addition, this study noted a significant negative correlation between ALT and HDL-c (16).

Both diastolic and systolic pressure was significantly positively correlated with ALP, ALT, AST, total bilirubin and direct bilirubin (P for trend ≤0.007). Furthermore, these were negatively correlated with TP and albumin (P ≤0.001). A similar finding was reported in a study from China (16). Obstruction of the blood flow due to the deposition of fibrin in the liver sinusoids might be the probable cause of the liver damage in participants with hypertension (17).

The likelihood of having an abnormal LFTs was greater among males than females. Previous studies have shown the association between male sex and abnormal LFTs (16, 18, 19). The sex difference may be explained by differences in body fat distribution due to the presence of estrogen in females (20).

Older age, with shorter duration of DM, was significantly associated with abnormal LFTs. In contrast to our finding and others (12, 16, 18), a study from Scotland (11) and Italy (21) reported younger age with shorter duration of DM as a factor for abnormal LFTs. This difference can be related to genetic variation as ethnic difference in NAFLD progression was reported to be determined by variants in genes (22). The inverse correlation between duration of DM and liver enzyme and bilirubin may be due to survival bias (people with more severe liver injury dying earlier) and treatment effect over time. Older subjects might be less consistent with the stringent lifestyle interventions usually prescribed as part of T2DM management.

Alcohol drink was significantly associated with abnormal LFTs. A similar finding has been observed by other studies (11, 12, 16, 18, 19). Alcohol drink might bring this effect by altering

lipid metabolism and direct toxicity. The breakdown of alcohol with cytosolic enzymes generates toxic metabolites such as acetaldehyde and highly reactive oxygen containing molecules evoking oxidative stress and inflammation(23).

Sweet drink was significantly associated with abnormal level of ALP (AOR=5.1(1.7-11.5)), ALT (AOR=6.2(1.7-16.0)) and TP (AOR=2.6(1.03-9.9)). The intake of sweetened beverages may affect insulin sensitivity, which results in impaired metabolism and hepatic steatosis (24). Butter consumption was significantly associated with elevated ALT (OR=1.4(1.01-2.6)) and AST (OR=2.1(1.2-3.4)). Evidences revealed that butter consumption is associated with increased risk of metabolic syndrome (25) which may induce liver function abnormality.

We have seen the inverse relationship between abnormal LFTs and coffee consumption. Coffee was reported to decrease abnormal LFTs in other studies (18, 26). Aromatic extracts (27) and chemical compounds such as cafestol, kahweol and chlorogenic acid (28, 29) has been suggested for their antioxidative function.

Strengths of this study include the comparative method we used. Consistent definitions were applied to all subjects, which we believe that help to reduce bias. Notably, based on literature search, it is the first study to report the prevalence of abnormal LFTs among T2DM patients in the study area. Despite the above strengths, our study has limitations. The study was a cross-sectional study design and thus, it is not possible to determine if diabetes preceded or followed the abnormal liver, nor is it possible to determine whether we observed chronic or transient LFTs impairment. Because of self-reporting measures of behavioral characteristics such as drinking, some error and resulting residual confounding by these covariates and others may not be excluded. Our study didn't use imaging methods and/or histology to ascertain the association of abnormal LFTs with NAFLD. This point is particularly important because it has been demonstrated that NAFLD could be present also in absence of elevated liver enzymes (30).

CONCLUSION AND RECOMMENDATIONS

In conclusion, the prevalence of abnormal level of LFTs in T2DM was higher than a control group. The mean values of the liver enzymes and bilirubin in patients with T2DM were significantly higher than that of a control group. Moreover, T2DM patients had lower level of TP and albumin in comparison to the control group. This difference indicates that T2DM may induce liver function impairment. The high prevalence of LFTs derangement in T2DM highlights the importance of requesting LFTs in these patients as they may harbor potentially treatable co-morbid illnesses. Health education about the potential risk of liver diseases and way of prevention shall be provided to T2DM patients as well. In addition, follow up study is required to ascertain the mechanism by which liver function is impaired.

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Abbreviations

ALD: *Alcoholic Liver Disease*

ALP: *Alkaline Phosphatase*

ALT: *Alanine Aminotransferase*

AST: *Aspartate Aminotransferase*

BMI: *Body Mass Index*

DM: *Diabetes Mellitus*

FBS: *Fasting Blood Sugar*

GGT: *Gamma Glutamyl Transferase*

HBV: *Hepatitis B Virus*

HCV: *Hepatitis C Virus*

HDL-c: *High Density Lipoprotein cholesterol*

IR: *Insulin Resistance*

LDL-c: *Low Density Lipoprotein cholesterol*

LFT: *Liver Function Test*

NAD: *Nicotinamide Adenine Dinucleotide*

NAFLD: *Nonalcoholic Fatty Liver Diseases*

TP: *Total Protein*

ULN: *Upper Limit of Normal*

TG: *Triglyceride*

TC: *Total Cholesterol*

T2DM: *Type 2 Diabetes Mellitus*

WC: *Waist Circumference*

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Data Availability

The data sets used and analyzed during the current study are available from corresponding author on a reasonable request.

Competing interest

All authors declared that there is no competing interest between all authors.

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Authors' contribution

All the authors contribute equally, starting from drafting of the proposal to preparation of the manuscript.

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Correlation of body mass index and waist/hip ratio with glycated hemoglobin in prediabetes

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ABSTRACT

Introduction:

Prediabetes (preDM) is a state of disordered glucose metabolism rather than a distinctive clinical entity representing an interim condition and a risk factor for the development of diabetes. Diagnosis of asymptomatic people to assess the risk for development of DM should be done in overweight or obese adults (BMI ≥ 25 kg/m^2) of any age along with person having one or more additional risk factors like physical inactivity, first degree relative with DM, high risk race/ethnicity, hypertension etc.

Objectives:

To correlate glycated hemoglobin (HbA_{1c}) levels with body mass index (BMI) and waist hip ratio (WHR) in prediabetic patients.

Materials & methods:

The present case control study was performed at Pt. B. D. Sharma PGIMS, Rohtak includes thirty prediabetic

patients of age group 20-40 years diagnosed on the basis of HbA_{1c} (5.7-6.4%). Thirty healthy and age matched control were taken. After taking written consent, they were subjected to physical examination and anthropometric measurements as per protocol and findings were noted. Venous blood sample was withdrawn for estimation of HbA_{1c} levels.

Results:

The correlation coefficient between BMI (27.01 ± 2.91 kg/m^2) and HbA_{1c} (5.94 ± 0.21%) is r = 0.583 with p value = 0.001 and between WHR (0.87 ± 0.38) & HbA_{1c} is r = 0.495 with p value = 0.005. Both BMI & WHR are positively correlated with HbA_{1c}.

Conclusion:

Obesity is a risk factor for glycation of hemoglobin & hence, it is an effective measure for prevention of prediabetes and diabetes.

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INTRODUCTION

Prediabetes (preDM) is a stage of disordered glucose metabolism rather than a distinct clinical entity and a risk factor for the development of diabetes along with an increase in cardiovascular and microvascular complications. The transition from preDM to diabetes may take years but may also be rapid. It is estimated that most individuals (up to 70%) with preDM eventually develop diabetes. The incidence is highest in individuals with combined impaired fasting glucose (IFG) & impaired glucose tolerance (IGT) and similar in those with isolated IFG (i-IFG) or isolated IGT (i-IGT) [1].

BMI ≥ 25 kg/m^2 is a major risk factor for development of prediabetes along with other risk factors like physical inactivity, first degree relative with DM, high risk race/ethnicity, women who delivered a baby weighing 9 lb or diagnosed with gestational DM, hypertension (HTN), HDL cholesterol (HDL-C) level of 35 mg/dL and a triglyceride (TG) level of 250 mg/dL, women with polycystic ovarian syndrome (PCOS), etc [2].

Figure 1 Formation of HbA1c [5]

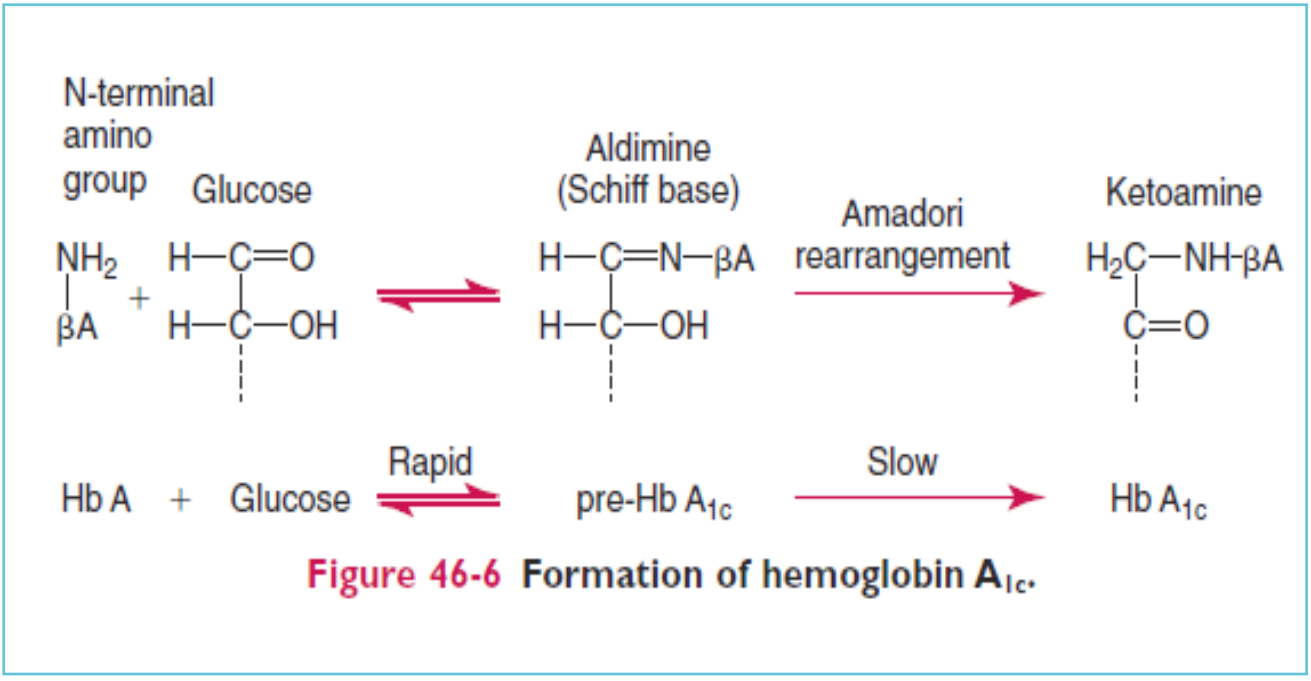


Figure 46-6 Formation of hemoglobin A_{1c}.

HbA_{1c} results from the nonenzymatic, irreversible concentration dependent covalent bonding of glucose to hemoglobin within the erythrocytes. Glycation occurs in a two step Maillard reaction. It involves the initial formation of a labile Schiff base which undergoes a subsequent Amadori rearrangement leading to formation of an Amadori product i.e., HbA_{1c}. Driven by the nucleophilic nature of the NH_2-terminal, amino group of hemoglobin condenses with glucose found in the erythrocyte. The cumulative amount of HbA_{1c} in an erythrocyte is directly proportional to the time dependant concentration of glucose within the erythrocyte [3,4] (Figure 1).

The concentration of HbA_{1c} correlates with the average blood glucose levels over the preceding three months. As a consequence of relationship between glycemia and HbA_{1c}, it is clear that there is a significant association between HbA_{1c} and various clinical outcomes. Moreover, HbA_{1c} is related to the risk of microvascular (in both type 1 and type 2 diabetes) as well as macrovascular (in type 1 diabetes) complications [6].

Thus, it is concluded that development of preDM is linked to environmental factors such as physical inactivity but the subsequent development of diabetes is affected by combination of genetic and environmental factors. Therefore efforts to prevent diabetes should be initiated prior to the development of preDM in order to obtain the maximum benefit [7].

MATERIALS & METHODS

The present study was conducted in the Department of Biochemistry, in collaboration with the Department of Medicine, Pt. B. D. Sharma PGIMS, Rohtak.

In the present study, 30 prediabetic patients diagnosed on the basis of HbA_{1c} levels were enrolled as cases. 30 healthy- and age-matched individuals were enrolled as controls.

Inclusion criteria

Patients of age group between 20-40 years satisfying the criteria of prediabetes based on HbA_{1c} were included in the study.

Criteria of prediabetes according to ADA is [8]

1. Impaired fasting glucose (IFG) with fasting plasma glucose levels of 100 to 125 mg/dL (5.6 to 6.9 mmol/L).
2. Impaired glucose tolerance (IGT) with plasma glucose levels of 140 to 199 mg/dL (7.8 to 11.0 mmol/L) 2-hour postprandial.
3. HbA_{1c} of 5.7 to 6.4%.

Exclusion criteria

- Patients with hemoglobin < 9 g/dL and any history suggestive of hemoglobinopathies.
- Patients with history suggestive of endocrine disorders like thyroid, adrenal and pituitary glands disorders.
- Patients with history suggestive of any drug intake affecting glucose metabolism.

Methodology

After getting written consent from the cases and controls, detailed history was taken and recorded in their respective proforma. They were subjected to physical examination and anthropometric measurements as per protocol and the findings were noted. Waist circumference (WC) was measured midway between the lowest point of rib cage and the superior border of iliac crest at the end of normal expiration with a stretch resistant measuring tape. Hip circumference (HC) was measured around the widest portion of the buttocks with the tape parallel to the floor. Waist hip circumference ratio (W/H Ratio) was calculated as WC in cm divided by HC in cm. Cut off values for WHR is 0.90 for men and 0.80 for women [9]. The weight and standing height of all study subjects were measured by using calibrated weighing scale and

stadiometer with a fixed vertical backboard and an adjustable head piece respectively. BMI can be calculated by the present weight in kg divided by height^2 in metre. BMI can be expressed in the units of kg/m^2 (Table 1).

Quetlet Index

BMI = weight (kg) / height^2 (in metre)

SAMPLE COLLECTION

For estimation of HbA_{1c}, 2 mL of blood was collected in EDTA anticoagulant vacutainer. Samples were processed & analysed on the same day. HbA_{1c} was determined by turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. Glycohemoglobin (HbA_{1c}) in the sample reacts with anti HbA_{1c} antibody to form soluble antigen antibody complexes. Since the specific HbA_{1c} antibody site is present only once on the HbA_{1c} molecule, complex formation does not take place. The polyhaptens react with excess anti HbA_{1c} antibodies to form an insoluble antibody polyhapten complex that can be measured turbidimetrically. [11]

STATISTICAL ANALYSIS

Primary outcome were calculated by applying Unpaired 't' test and secondary outcome were obtained by using two-tailed Pearson correlation between variables of prediabetic cases and controls by using the statistical package (IBM SPSS 20). Data were considered to be significant if $p < 0.05$ and highly significant with $p < 0.001$.

Table 1 WHO classification of BMI grading [10]

BMI (kg/m^2)	Classification
< 18.5	Underweight
18.5-24.9	Normal Weight
25.0-29.9	Overweight
30.0-34.9	Class I Obesity
35.0-39.9	Class II Obesity
≥ 40.0	Class III Obesity

Table 2 Data of study group

S. no.	Data	Cases (n = 30) mean ± SD	Control (n = 30) mean ± SD	p value
1.	BMI (kg/m^2)	27.01 ± 2.91	24.16 ± 1.25	**0.001**
2.	WHR	0.87 ± 0.38	0.80 ± 0.38	**0.001**
3.	HbA_{1c} (%)	5.94 ± 0.21	5.24 ± 0.32	**0.001**

RESULTS & OBSERVATIONS

In the present study, out of 30 cases, 11 (37%) were normal, 13 (43%) were overweight and 6 (20%) were obese. Out of 30 controls, 20 (67%) were normal while 10 (33%) were overweight. 63% of cases had increased BMI that is much higher than 33% of controls having increased BMI (Table 2).

WHR showed statistical significant difference between cases (mean 0.87 ± 0.38) and controls (mean 0.80 ± 0.38) with p value = 0.001. Out of 30 cases, 27 (90%) had increase WHR while 3 (10%) had normal WHR. Out of 30 controls, 13 (43%) had increase WHR while 17 (57%) had normal WHR. So, 90% of cases had increase WHR in comparison to 43% of controls.

TWO-TAILED PEARSON'S CORRELATION BETWEEN PARAMETERS

In the present study, it was found that HbA_{1c} had positive correlation with BMI as well as WHR (Table 3, Figure 2A and 2B).

DISCUSSION

Development of preDM is linked to environmental factors such as physical inactivity but the subsequent development of diabetes is affected by combination of genetic and environmental factors [7]. Adverse environmental factors or disease can cause cells to fail to respond to insulin leading to insulin resistance (IR). Once IR develops, the body cells fail to respond to insulin and are unable to use it effectively leading to development of IGT. When the condition develops further, apoptosis of islet cells occurs and glucose metabolism is disrupted leading to clinical DM [2].

In the present study, 63% of cases had increased BMI in comparison to 33% of controls having increased BMI. HbA_{1c} had significant positive correlation with BMI and WHR. Our observation is supported by study done by Li et al and Abtahi et al.

Li et al reported a positive correlation between HbA_{1c} and BMI in preDM. It is found that oxidative stress is a key determinant of glycation of hemoglobin leading to increase HbA_{1c} levels with elevated oxidative stress in nondiabetic subjects. Oxidative stress affects HbA_{1c} level through two ways. Firstly, the glycation of hemoglobin is a two step Maillard reaction involving the initial formation of a labile Schiff base and a subsequent Amadori rearrangement. Oxidative stress facilitates the autoxidation of glucose to dicarbonyl intermediates in an early step of the Maillard reaction and thus enhancing the glycation of proteins. Secondly, oxidative stress results in insulin resistance within adipose and skeletal muscle tissues and subsequent development of hyperglycemia which will further increase the oxidative stress [12].

Lipid peroxidation also affects glycation of hemoglobin independent of glucose concentration. Therefore, oxidative stress may partly explain the discordance between HbA_{1c} levels and blood glucose diagnosing diabetes and preDM.

Table 3 Correlation of HbA1c with different parameters

S. no.	Parameters	Correlation coefficient (r)	p value
1.	HbA_{1c} Vs BMI	0.583	**0.001**
2.	HbA_{1c} Vs WHR	0.495	**0.005**

Figure 2A Graph showing the correlation between HbA_{1c} and BMI

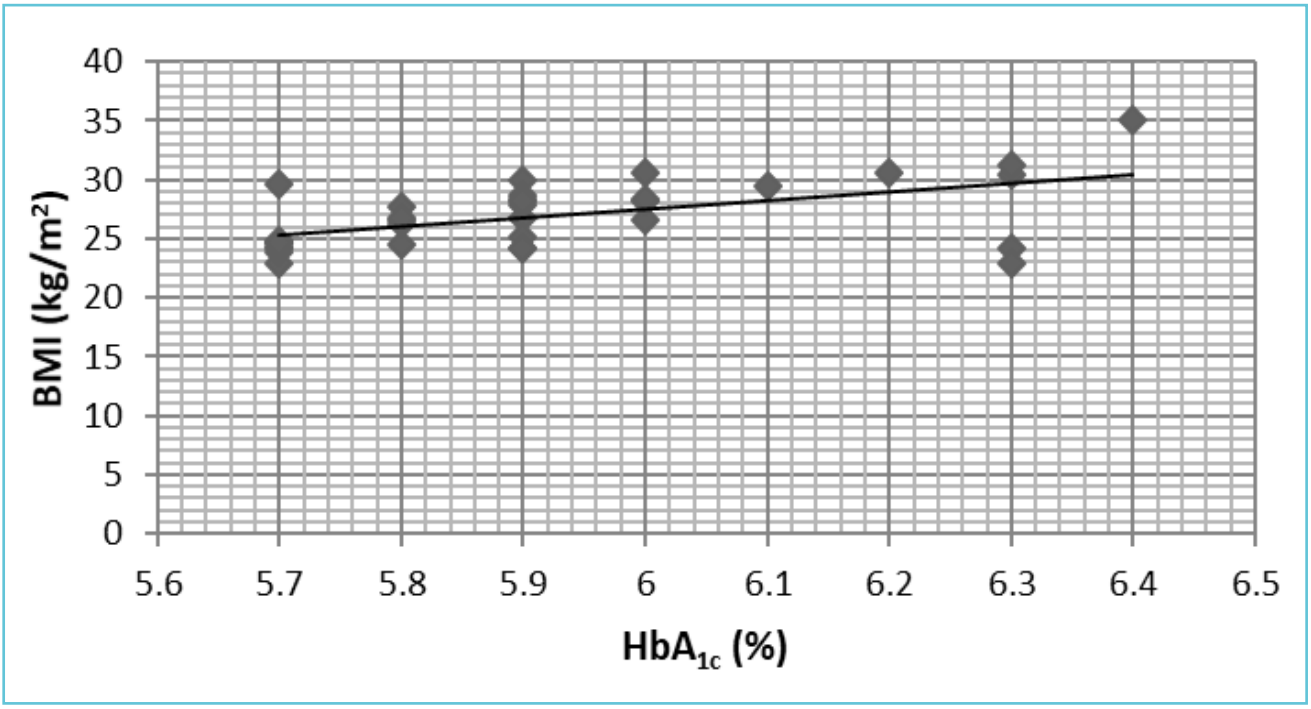
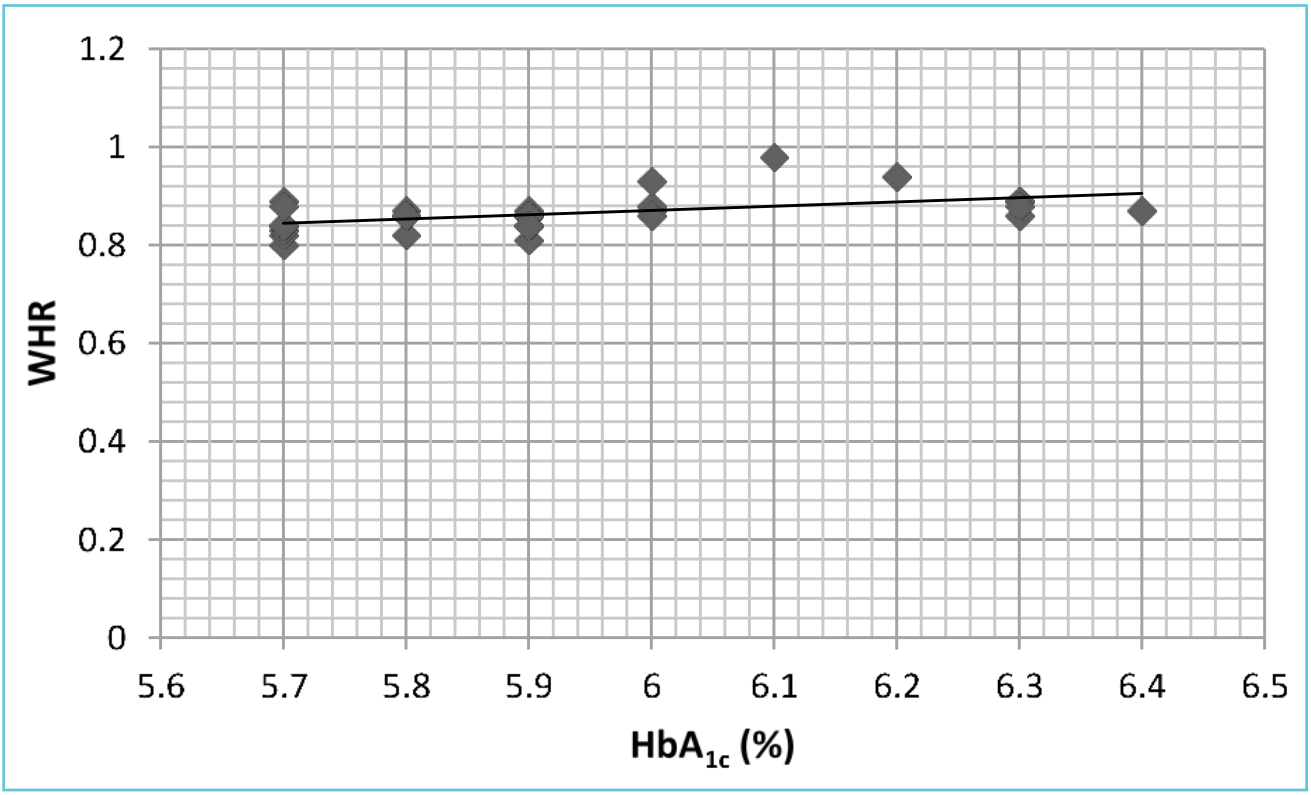


Figure 2B Graph showing the correlation between HbA_{1c} and WHR



Obesity has been reported as a strong independent predictor of systemic oxidative stress. Thus the association between BMI and HbA_{1c} is mediated by oxidative stress.

Obesity can induce systemic oxidative stress leading to increased glycation of hemoglobin independent of glucose levels. Thus HbA_{1c} concentration may be disproportionately elevated at a given glycemic level in obese subjects. So, HbA_{1c} cannot reflect the real concentration of glucose in obese subjects [13,14].

Abtahi et al observed that the prevalence of preDM was higher in obese person having higher range of waist circumference, WHR and BMI. Body weight is determined by many factors such as genetic, behavioral, cultural, socio-economic, physical inactivity, diet and psychosocial factors. Excess body weight is a risk factor for a variety of health hazards like DM, preDM and cardiovascular disease. It was concluded that people with lower BMI are less susceptible for development of DM & preDM and obese people have higher prevalence of abnormal blood glucose levels [15].

Various studies have shown that one to three quarters of subjects with IGT develop diabetes within a decade of discovery of IGT and annual progression rates from IGT to diabetes range from 1 to 10%. Thus by slowing the progression, the incidence of diabetes would be reduced and the onset of its complications would be prevented or delayed [16-19]. Early intervention is required to improve the progression of the complications and reduce the cost of disease in the long term. A sedentary lifestyle increases the risk for development of IR. Energy expenditure of 500 kcal/week decreases risk of developing type 2 DM by 6%. According to one study, vigorous exercise at least once a week reduces the risk of type 2 DM in women by 33% [2].

According to the Da Qing study exercise advice (with or without dietary advice) appears more effective than dietary advice alone. Exercise increases insulin mediated glucose disposal in muscles. Although in humans low dietary fat content does not influence insulin mediated glucose disposal but hypocaloric diet leading to weight loss is associated with improved insulin mediated glucose disposal and reduction of glycemia. Thus, these interventions lead to reduce IR, slows the progression of glucose intolerance and arrests or delays β cell deterioration [20].

CONCLUSION

BMI and WHR has a role in glycation of hemoglobin and obesity is a preventable risk factor for the development of prediabetes and its further consequences.

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Hyperuricemia and its association with cardiovascular disease risk factors in type two diabetes mellitus patients at the University of Gondar Hospital, Northwest Ethiopia

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ABSTRACT

Background:

Hyperuricemia is associated with cardiovascular disease (CVD) that presents in diabetes mellitus patients. Therefore, the aim of this study was to appraise the serum uric acid and its association with CVD risk factors among diabetes mellitus patients.

Methods:

A cross-sectional study was carried out at the University of Gondar hospital from February to March, 2018. A total of 384 study participants were selected by systematic random sampling technique. Five milliliter blood sample was collected and analyzed using Mindray BS-200E machine. The data was analysed into SPSS version 20. Logistic regression model was used to investigate associated factors. A p-value <0.05 was considered statistically significant.

Results:

The prevalence of hyperuricemia among type 2 diabetic patients was 31.5%. The serum uric acid concentration was higher among male (33.1%) compared to female (28.9%). Elevated systolic blood pressure (AOR: 4.4, 95%CI: 2.1-9.3), family history of DM (AOR: 1.5, 95%CI: 1.2-2.5) and BMI ≥ 25 Kg/m^2 (AOR: 1.4, 95%CI: 1.1-3.7) were significantly associated with hyperuricemia. Increased BMI (52.4%), high waist circumference (63.0%) and elevated systolic blood pressure (58.2%) were the major CVD risk factors.

Conclusion:

The prevalence of hyperuricemia was high in type 2 diabetes patients. The major predictors of CVD risk factors were elevated systolic blood pressure, family history of DM and BMI ≥ 25 Kg/m^2 which lead to early diagnosis and treatment for hyperuricemia. Lastly, CVD risk factors are essential to reduce the disease among type 2 diabetic patients.

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Abbreviations

AOR: *Adjusted Odds Ratio*

ABCG2A: *TP Binding Cassette transporter sub family G member 2*

ADP: *Adenosine Diphosphate*

ALT: *Alanine Aminotransferase*

ATP: *Adenosine Triphosphate*

BMI: *Body Mass Index*

BP: *Blood Pressure*

CE: *Cholesteryl Esterase*

CI: *Confidence Interval*

COR: *Crude Odds Ratio*

CVD: *Cardiovascular Disease*

DM: *Diabetes Mellitus*

FBG: *Fasting Blood Glucose*

FHDM: *Family History of disease*

HDL: *High Density Lipoprotein*

HUA: *Hyperuricemia*

IR: *Insulin Resistance*

LDL: *Low Density Lipoprotein*

MetS: *Metabolic Syndrome*

MSU: *Monosodium Urate*

SUA: *Serum Uric Acid*

tCho: *Total Cholesterol*

T2DM: *Type 2 Diabetes Mellitu*

TG: *Triglyceride*

UA: *Uric Acid*

VLDL: *Very Low Density Lipoprotein*

WC: *Waist Circumference*

XOR: *Xanthine oxido-reductase*

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BACKGROUND

Uric acid (UA) is a final enzymatic product of purine metabolism in humans [1] and it is regulated by the xanthine-oxidoreductase enzyme, which converts hypoxanthine to xanthine and xanthine to uric acid [2]. An elevated concentration of UA is associated with a variety of cardiovascular conditions [3]. The balance between the intake endogenous synthesis, excretion ratio and metabolism of purines determines the concentrations of Serum Uric Acid (SUA). The alteration of any of these factors could cause hyperuricemia (HUA), which defined as a SUA concentration >6.8 mg/dL[4]. Currently, the prevalence of HUA is potentially attributed to recent shifts in diet and lifestyle, improved medical care and increased long life [5].

Developed countries tend to have a higher burden of gout than developing countries. Some ethnic groups are particularly vulnerable to gout, supporting the importance of genetic predisposition. Socioeconomic and dietary factors, as well as co-morbidities and medications that can impact UA levels and/or facilitate monosodium urate (MSU) crystal formation, are also important in determining the risk of developing gout [6].

Recently, SUA has received attention as a potential biomarker dependently predicting the development of hypertension, diabetes mellitus (DM), and chronic kidney disease [7]. A close relationship exists between plasma UA levels and glucose utilization in type 2 diabetes mellitus (T2DM) [8], which results from a defect in insulin secretion or action, almost always with a major contribution from insulin resistance (IR) [9]. T2DM is a corollary of the interaction between a genetic predisposition, behavioral and environmental risk factors. Obesity and physical inactivity are the main non-genetic determinants of T2DM although, the genetic basis of the disease has yet to be identified [10]. The strong relationship between UA and T2DM is due to the development of renal dysfunction in T2DM [11].

There were studies that showed a clear relationship of increased UA levels with hypertension, metabolic syndrome (MetS), abdominal obesity, endothelial dysfunction, inflammation, subclinical atherosclerosis and an increased risk of cardiovascular events [12]. Some other factors can also induce HUA such as hypertension, possibly by urate reabsorption, which is caused by decreased renal blood flow [13]. Dyslipidemia may also cause HUA through a negative effect on renal function [14]. According to data from the National Health and Nutrition Examination Survey (NHANES) 2007–2008, the prevalence of HUA was 21% in American adults, reaching 26% in African Americans. Recently, the prevalence of HUA has been increasing [15]. Evidence has supported the association of high level of UA with MetS, T2DM and CVD [16].

Some of the recognized risk factors of CVD are high blood pressure, rapid acculturation and step up in economic conditions, economic transition, increased tobacco use, high blood lipids, physical idleness, over-weight and obese, DM and poor dietary habit [15]. Hyperglycemia and lipid metabolism disorder is also linked to a greater risk for vascular problems, kidney disease, nerve and retinal damage resulting in challenges in managing the disease adequately, especially in the presence of immune suppression, and predisposes individual to premature mortality. Moreover, this has cost and social implications for patients, their families, communities and the healthcare system. Currently, HUA in T2DM patients has been less well investigated in sub-Saharan Africans. Until now, the pathogenic role of UA in the development of the MetS is not complete, therefore, the aim of the study was to assess the current burden of HUA and its association with CVD risk factors among T2DM patients at the University of Gondar Hospital.

METHODS AND MATERIALS

Study design, period and area

Institution-based cross-sectional study was conducted from February to March, 2018 at the University of Gondar Hospital DM clinic, Gondar, Ethiopia. The University of Gondar Hospital is one of the biggest hospitals in Amhara region that provides health service, acts as a referral center for other district hospitals and has about 400 beds.

It is expected to deliver health service for about five million people in Northwest Ethiopia. As a teaching hospital, it plays an important role in teaching, research and community service.

According to the 2007 census, Gondar town has a total population of 323,900 [17].

Population

The source population was all T2DM patients who have access to be served at the University of Gondar Hospital. Moreover, the study population were all individuals with T2DM who visited the hospital during the study period and fulfilled eligibility criteria.

Inclusion and exclusion criteria

All T2DM patients > 18 years old who were willing to participate in this study were included. Pregnant women, severely ill individuals and patients on drugs known to have an effect on UA level except for anti-diabetic therapy and patients taking lipid lowering drugs were excluded from the study.

Operational definition

Study participants were classified as underweight (BMI<18.5 Kg/m^2), normal weight (18.5-24.9 Kg/m^2), overweight (BMI =25-29.9Kg/m^2) and obese (BMI ≥30Kg/m^2)(18). Waist circumference (WC) >88 centimeter for female and WC >101 centimeter for male was taken as high WC [18] Systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or current use of blood pressure-lowering medication was used to define hypertension [19]. The interpretation of test results for fasting blood sugar (FBS), UA and lipid profiles was based on the reference range recommended by the manufacturers instruction were considered as normal.

Sample size determination and sampling technique

Single population proportion formula was used by considering the proportion of 50% prevalence among T2DM. 5% desired precision and 95% confidence interval (CI) resulting in a total sample size of 384. The study participants were selected using a systematic random sampling technique.

Data collection and laboratory methods

Socio-demographic characteristics and clinical data were collected by trained nurses using a semi-structured questionnaire. In addition to that, trained laboratory technologists collect and analyzed the blood sample. Anthropometric measurement (weight, height) was measured according to WHO stepwise approach guideline. Height was measured to the nearest 0.5 cm using standiometer and weight was recorded to the nearest 0.1 kg with the patient wearing light clothes using a balance. BMI was calculated as weight divided by height squared (kg/m^2) [18].

Blood pressure was measured by nurses using an analogue sphygmomanometer. Five milliliter fasting venous blood sample was collected using serum separator test tube by following aseptic blood collection procedure. Serum glucose, lipid profiles and UA were measured by using Mindray BS-200E chemistry analyzer (Shenzhen Mindray Bio-Medical electronics Co. Ltd, China).

Data analysis and interpretation

Data was checked for its completeness, clarity and edited for its consistency and the data was entered to SPSS version 20 statistical package for analysis. Descriptive statistics were used to summarize the frequency distributions. Logistic regression analysis was used to determine the association between dependent and independent variables.

Variables with P value < 0.25 in binary logistic regression model were included into the multivariable analysis model to identify independent predictor variables for abnormal serum uric acid concentration. In addition, Pearson's correlation was used to determine the correlation between independent variables and serum UA.

Ethical consideration

Ethical clearance was obtained from the Research and Ethical Review Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Permission letter was also taken from clinical director of the Hospital and head of the DM clinic. To ensure the confidentiality of the study participant's information, anonymous typing was applied, so that the name and any identifier of the participants were not written on the questionnaire.

RESULTS

Serum uric acid level according to socio-demographic characteristics of study participants

A total of 384 study participants were enrolled and the response rate obtained was 99.1%. In this study, a majority of 60.4% (n=232) of the study participants were males. The mean age of the study participants was 55.74 ± 9.05 years with a range of 36 to 88 years. 95% (n=365), 96.1 % (n=370), and 59.1% (n=227) study participants

Table 1 Serum uric acid level of the study participants according to socio-demographic characteristics

Variables	Category	N (%)	Uric acid level, N (%)		P-value
			Hyperuricemia	Normouricaemia	
Sex	Male	232(60.4)	77(33.2)	155(66.8)	0.382
	Female	152(39.6)	44(28.9)	108(71.1)	
Age	36-45	46(12.0)	14(30.4)	32(69.6)	**0.001***
	46-55	148(38.5)	28(18.9)	120(81.0)	
	56-65	139(36.2)	52(37.4)	87(62.6)	
	66-75	41(10.7)	24(58.5)	17(41.5)	
	76-88	10(2.6)	3(30.0)	7(70)	
Marital status	Unmarried	12(3.1)	7(58.3)	5(41.6)	**0.041***
	Married	370(96.1)	113(30.5)	257(69.4)	
Educational level	Literate	104(27.1)	33(31.7)	71(68.2)	0.898
	Illiterate	277(72.1)	86(31.0)	191(69.0)	
Resident	Urban	365(95.1)	115(31.5)	250(68.5)	0.995
	Rural	19(4.9)	6(31.5)	13(68.5)	
Occupation	Employed	276(59.1)	89(32.2)	187(67.8)	0.524
	Unemployed	104(39.8)	30(28.8)	74(71.1)	

Table 2 Serum uric acid level according to clinical characteristics of study participants

Variables	Category	N (%)	Uric acid level, N (%)		P-value
			Hyperuricemia	Normouricaemia	
FHDM	Yes	121(31.5)	57(47.1)	64(52.9)	0.001*
	No	263(68.5)	64(24.3)	199(75.6)	
Hypertension	Present	118(30.8)	82(69.4)	36(30.6)	0.001*
	Absent	266(69.2)	39(14.6)	227(85.4)	
WC	High	92(24.0)	58(63.0)	34(37.0)	0.001*
	Normal	292(76.0)	63(21.5)	229(78.4)	
BMI	Normal	239(62.2)	46(19.2)	193(80.7)	0.001*
	High	143(37.2)	75(52.4)	68(47.5)	
SBP	High	79(20.6)	46(58.2)	33(41.7)	0.001*
	Normal	305(79.4)	75(24.6)	230(75.4)	
DBP	High	56(14.6)	36(64.2)	20(35.8)	0.26
	Normal	328(85.4)	85(25.9)	243(74.1)	
Duration of DM	<5 yr	248(64.6)	63(25.4)	185(74.6)	0.02*
	6-10 yr	100(26)	44(44.0)	56(56.0)	
	>10 yr	36(9.4)	14(38.8)	22(61.2)	
Physical activity	No	304(79.2)	100(32.9)	204(67.1)	0.28
	Yes	80(20.8)	21(26.2)	59(73.8)	
Alcohol	Yes	88(22.9)	31(35.2)	57(64.7)	0.393
	No	296(77.1)	90(30.7)	206(69.2)	
Coffee	Yes	265(69.0)	81(30.5)	184(69.5)	0.552
	No	119(31.0)	40(33.6)	79(66.3)	

*FHDM: Family History of Diabetes mellitus; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; WC: Waist Circumference; BMI: Body Mass Index; *P-value < 0.05, statistically significant association.*

Table 3 Serum uric acid level and biochemical parameters of the study participants

Variables	Category	N (%)	Uric acid level, N (%)		P-value
			Hyperuricemia	Normouricaemia	
TG (mg/dl)	High	199(51.8)	85(42.7)	114(57.3)	0.001
	Normal	185(48.1)	36(19.4)	149(80.6)	
tCho (mg/dl)	High	171(44.5)	66(38.6)	105(61.4)	0.007
	Normal	213(60.1)	55(25.8)	158(74.2)	
LDL (mg/dl)	High	131(34.1)	73(55.7)	58(44.3)	0.001
	Normal	253(65.8)	48(18.9)	208(81.1)	
HDL (mg/dl)	Low	77(20.0)	61(79.2)	16(20.8)	0.001
	Normal	307(79.9)	60(19.5)	247(80.5)	
FBS (mg/dl)	High	362(94.2)	118(32.6)	224(61.8)	0.063
	Normal	22(5.7)	3(13.6)	19(8)	

TG: Triglyceride; FBG: Fasting Blood Glucose; tCho: Total Cholesterol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; mg/dl: milligram per deciliter.

were urban dwellers, married and employed, respectively. The prevalence of HUA was 31.5% (n=121) with 95% CI, 27.3-36.2. The serum uric acid concentration was higher among male study participants compared to female (33.1% versus 28.9% respectively) and the prevalence was also higher among ≥45 years age group (31.8%) (Table 1).

Serum uric acid level according to clinical characteristics of study participants

The prevalence of HUA was higher among study participants with a family history of diabetes (47.1%). Higher prevalence of HUA was determined among patients with ≥5-year duration of diabetes (42.6%), overweight (BMI: 25–29.9 Kg/m^2) 52.4% (n= 49), hypertensive (69.4%) T2DM patients.

The high percentage of abnormal serum uric acid concentration was determined among study participants with central obesity (63.0%), with elevated SBP (58.2%), and with family history of DM (47.1%) (Table 2).

Serum uric acid level and biochemical parameters of study participants

The HUA concentration was determined among 42.7% (n=85) study participants with hypertriglyceridemia, among 79.2% (n=61) with reduced HDL, and in 32.6% (n=118) with hyperglycemic (Table 3).

Correlations of selected cardiovascular disease risk factors with serum uric acid level

The Pearson's correlation coefficient had indicated significantly positive correlation between

HUA and biochemical parameters like TG (r= 0.3, p value=0.001), FBG (r=0.3, p value=0.063), tCho (r=0.3, p value=0.007), and significantly negative correlation with HDL (r=-0.3, p value=0.001). In addition to that, some anthropometric parameters including BMI (r=0.1), WC (r=0.3) and SBP (r=0.2) have significantly positive correlation with HUA (Table 4).

The association between serum uric acid and cardiovascular disease risk factors among type 2 Diabetes Mellitus patients

In this study, T2DM patients with a higher Systolic BP (AOR = 4.4, 95% C.I (2.1-9.3), WC (AOR = 3.7, 95% CI (1.6-8.8), and with high BMI (AOR = 1.4, 95% C.I (1.1-3.7) were considerably associated with hyperuricemia (Table 5).

The prevalence of cardiovascular disease risk factors among T2DM patients

About, 29.6% (n=121) of the study, participants have single CVD risk factor, that is followed by two CVD risk factor 24.8% (n=93). At least one CVD risk factor was observed in 97.4% (n=374) of the study participants. Hypertension 58.6%; dyslipidemia 64.9%; overweight: 37.2% and central obesity: 24.0% were selected CVD risk factors (Figure 1).

Table 4 Pearson's correlation of cardiovascular disease risk factors with serum uric acid level at University of Gondar Hospital, 2018

Parameters	Mean ± SD	Correlation coefficients	P-value
TG (mg/dl)	272.2 ± 194.6	0.3	0.001*
FBG (mg/dl)	192.8 ± 66.9	0.3	0.063
tCho (mg/dl)	226 ± 152.5	0.3	0.007*
HDL (mg/dl)	57.4 ± 19.8	-0.3	0.001*
LDL (mg/dl)	97.8 ±52.3	0.3	0.001*
SBP (mmHg)	131.6 ± 13.8	0.2	0.001*
DBP (mmHg)	81.9 ± 8.6	0.2	0.001*
WC (cm)	94.3 ± 9.4	0.3	0.001*
BMI (kg/m^2)	25.4 ± 12.3	0.1	0.003*

TG: Triglyceride; FBG: Fasting Blood Glucose; tCho: Total Cholesterol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; WC: Waist Circumference; BMI: Body Mass Index; mmHg: millimeter mercury; mg/dl: milligram per deciliter; kg/m^2: Kilogram per meter square
** P-value < 0.05 is statistically significant.*

Table 5 Logistic regression analysis of the association of serum uric acid and cardiovascular disease risk factors among T2DM patients

Variables		N (%)	Uric acid level		COR (95% CI)	AOR	P-value
			Hyper-uricemia	Normouri-caemia			
Sex	Male	232 (60.4)	77	155	1.2 (0.7-1.9)	-	-
	Female	152 (39.6)	44	108	1	-	
Age	>45	336 (87.5)	107	229	1.1 (0.5-2.2)	-	-
	<45	48 (12.5)	14	34	1	-	
Duration of DM	0-5	248 (64.6)	63	185	1	1	**0.002***
	6-10	118 (30.8)	53	65	2.3 (1.5-3.8)	2.4 (1.4-4.2)	
	>10	18 (4.6)	5	13	1.1 (0.3-3.2)	-	
Hypertension	Present	118 (30.8)	82	36	13.2 (7.8-22.2)	13.9 (7.9-24.6)	**0.001***
	Absent	266 (69.2)	39	227	1	1	
Systolic BP	High	79 (20.6)	46	33	4.2 (2.5-7.1)	4.4 (2.1-9.3)	**0.03***
	Normal	305 (79.4)	75	230	1	1	
Diastolic BP	High	56 (14.6)	36	20	5.1 (2.8-9.3)	2.2 (0.8-5.6)	0.089
	Normal	328 (85.4)	85	243	1	-	

Family history DM	Yes	121 (31.5)	57	64	2.7 (1.7-4.3)	1.5 (1.2-2.5)	0.05
	No	263 (68.5)	64	199	1	1	
WC	High	92 (24)	58	34	6.2 (3.7-10.2)	3.7 (1.6-8.8)	**0.001***
	Normal	292 (76)	63	229	1	1	
BMI	High	143 (37.4)	75	68	4.6 (2.9-7.3)	2.0 (1.1-3.7)	**0.03***
	Normal	239 (62.6)	46	193	1	1	
Alcohol drinking habit	Yes	88 (22.9)	31	57	1.2 (0.7-2.0)	-	-
	No	296 (77.1)	90	206	1	-	
Coffee drinking habit	Yes	265 (69)	81	184	0.8 (0.5-1.3)	-	-
	No	119 (31)	40	79	1	-	
Physical activity	Yes	80 (20.8)	21	59	0.7 (0.4-1.2)	-	-
	No	304 (79.2)	100	204	1	-	

*WC: Waist Circumference; BMI: Body Mass Index; DM: Diabetes Mellitus; BP: Blood Pressure; COR: Crude Odds Ratio; AOR: Adjusted Odds Ratio; * P value < 0.05 is statistically significant.*

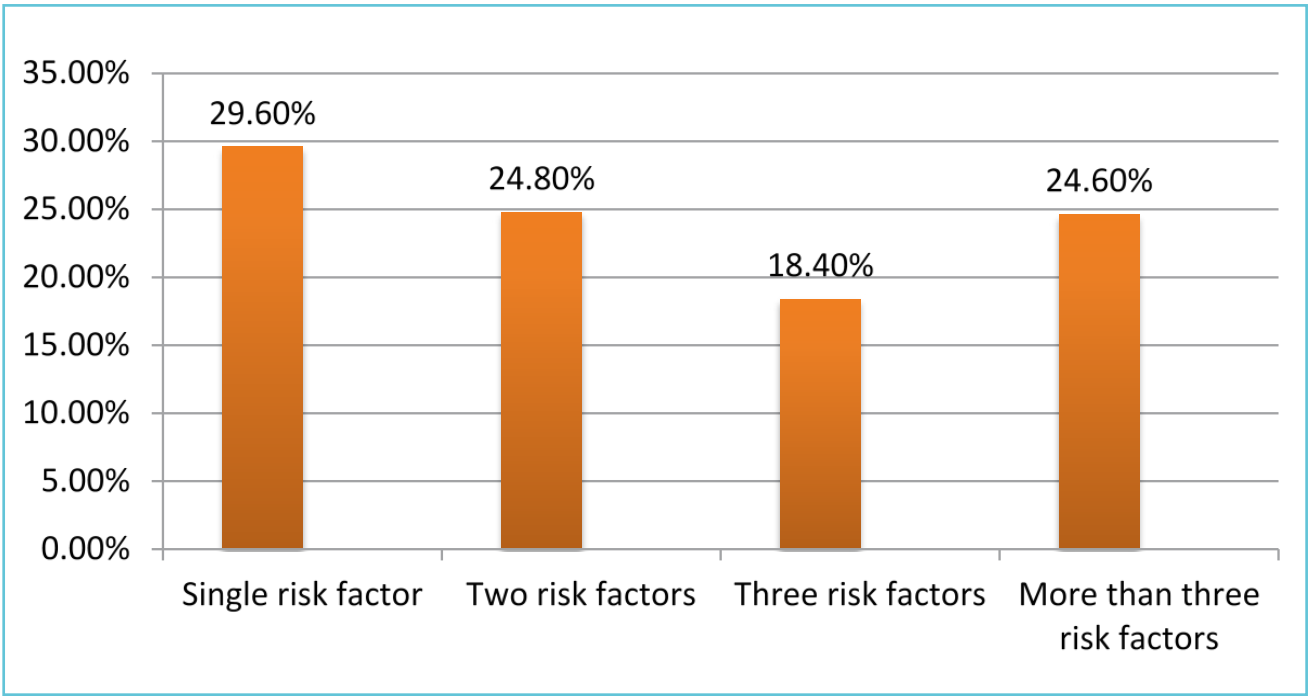
DISCUSSION

A previous study has reported that moderately raised levels of SUA have been considered as a simple biochemical defect with little clinical significance. However, recently, it has become increasingly clear that moderately elevated SUA levels are independently associated with increased cardiovascular morbidity and mortality in T2DM patients [20].

The main finding of this study was high prevalence of HUA concentration among T2DM patients. There was significant association between HUA and the various types of the CVD risk factors, and an increase in number of each CVD risk factor among the study participants.

In this study, the prevalence of HUA among T2DM patients was 31.5%. The magnitude of HUA that was reported by Wang J et al. from China (32.2%), Shah P et al. from Egypt (32.0%),

Figure 1 The overall prevalence of cardiovascular disease risk factors among T2DM patients at University of Gondar Hospital, northwest Ethiopia, 2018



Woyesa et al. from Hawassa, Ethiopia (33.8%)[21-23] was comparable to our finding. In contrast to the current finding, low prevalence of HUA was reported by Moulin SR et al. from Angola (25.0%) and Mundhe et al. from India (25.3%)[24, 25], and much less prevalence was reported from US (21.0%)(26). The variation in prevalence across studies might be due to the different life style, and the existence of ethnic variation between people in different countries [27].

The magnitude of HUA in males was higher than females in our study, which was supported by the study conducted in Nigeria and India [25, 28]. These sex differences of SUA levels have been attributed to the influence of sex hormones [29], due to the mechanism of estrogen in promoting UA excretion [30].

The other possible explanation for this, could be that males are more exposed to alcohol consumption [29] since, beer contains large amounts of purine [31] and the increased renal ATP binding cassette transporter sub family G member 2 (ABCG2) expression in men compared with women. The expression of the ABCG2 protein induces HUA through the reabsorption of urate [32].

In contrast to our finding, the prevalence of HUA from China, by Wang et al [23], was high among female study participants. The difference might be due to the ethnic difference of the study participants across countries.

On the other hand, the prevalence HUA in Nigeria [33] and Taiwan [34] were comparable in both genders. Beyond dietetic factors, HUA can also be related to the genetic predisposition for higher urate reabsorption in the kidneys.

Previous studies had shown that the ABCG2 protein, a UA transporter, shows differences in its expression and function by ethnicity [27].

In our study, age greater than 45 years had high prevalence of HUA, which was similar to the study conducted in Hawassa, Ethiopia [22] and

China [23]. The reason that might occur is that the effect of diuretics [35], due to ABCG2 protein, which increases as age increases and renal complications during aging [27].

The magnitude of hypertension (58.6%) in our study was comparable with the study conducted in Himalayan areas (61.5%)(36), and its prevalence was lower from Northern Catalonians (74.5%) [37]. In addition to that, the overall magnitude of dyslipidemia in our study was lower compared to the study in North Catalonia (77.7%) [37].

Similar study conducted in North Catalonia showed the different types of specific CVD risk factors, which include high BMI (>25 kg/m^2) (60.9%) and hypertension (80.3%), which was higher than the current study.

On the other hand, hypertriglyceridemia (35.6%) and lower HDL (19.5%) were lower compared with our study. The possible explanation might be due to the life style, ethnicity and cultural difference between those two regions [37].

The simultaneous presence of three or more CVD risk factors in the current study was observed in 24.6% of the study participants. This was much less from the study conducted in North Catalonia (91.3) [37].

The occurrence of at least one CVD risk factor in our study was observed in 97.4%. In this study, the duration of DM and family history of diabetes had statistically significant association with HUA, which is in line with the finding reported from India (38).

The possible mechanisms to explain these associations are the use of diuretics [35] or impaired renal function [39]. Genetic predisposition could be one of the reasons for the effect of HUA because of the gross overproduction of UA which results from the inability to recycle either hypoxanthine or guanine in patients genetically deficient in Hypoxanthine-guanine phosphoribosyl transferase (HPRT), inducing a lack of feed-back control of purine synthesis, which accompanied by rapid catabolism of purines to UA [40].

Increased SBP had significantly associated with HUA, which was supported by the study conducted in Black Africans [24].

The possible factor might be the use of antihypertensive agents, such as diuretics, which are known to increase HUA [35] and T2DM with hypertensive patients showed a significant association with HUA compared to non-hypertensive participants which is supported by a study on Black Africans, hence, anti-hypertensive therapy contributes significantly increases HUA [41].

In this study, high WC and high BMI (>25Kg/m^2) were significantly associated with HUA. This finding was supported by studies conducted in Nigerian, China and India [23, 25, 28]. The possible reason might be as a result of increase in Xanthine oxidoreductase (XOR) in obese individuals catalyzes oxidative hydroxylation of hypoxanthine to xanthine to uric acid (35).The level of HUA, accompanied with a significantly correlation with LDL, TG, TC, and HDL levels, in our study, which is agreed with study conducted in US [42]. Evidence also supported that dyslipidemia may cause HUA a negative effect on renal function [14].

Anthropometric measurements, such as high BMI, high SBP, high WC, as well as biochemical parameters, such as FBG and TG, were positively correlated with HUA.

The current study showed that, low HDL had a negative correlation with HUA, which were supported by the studies conducted in Ethiopia, China, Taiwan and India [22, 23, 34, 43], and a number of pathophysiological mechanisms have been explained to these associations including insulin resistance (IR) [44], the use of diuretics [35] or impaired renal function [39].

Patients who have IR, secrete larger amounts of insulin to maintain an adequate glucose metabolism and the kidney responds to the high insulin levels by decreasing UA clearance, probably linked to insulin-induced urinary sodium retention [45].

Due to these, the kidney has been implicated as the potential link between IR and compensatory hyperinsulinemia and the development of HUA.

The limitation of this study was cross-sectional nature of the study design that does not allow the establishment of causal relationship.

CONCLUSION AND RECOMMENDATION

The prevalence of hyperuricemia was high in type 2 diabetes patients. The major predictors of CVD risk factors were elevated systolic blood pressure, family history of DM and BMI ≥ 25 Kg/m^2.

There was significantly positive correlation of HUA with hypertriglyceridemia, hypercholesterolemia, high LDL, high WC and increased BMI. Therefore, early diagnosis and treatment for hyperuricemia and CVD risk factors are essential to reduce the disease among type 2 diabetic patients.

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Availability of data and materials

All relevant data supporting the conclusion are within the paper. The datasets used for this manuscript are available from the corresponding author on reasonable request.

Authors' contributions

All authors participated in data collection, analysis, and interpretation of the result, write up and reviewed the initial and final drafts of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declared that there is no competing interest.

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Atypical hemolytic uremic syndrome: genetic landscape challenge

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CASE REPORT

An obese and current smoker 23-year-old woman presented to the Emergency Department with abdominal pain, two episodes of vomiting and watery non-bloody diarrheic depositions. She indicated she had not urinated for ten hours. The patient had come the previous day complaining of intense headache, being prescribed dexketoprofen, diazepam and metoclopramide, as she had had previous episodes of whiplash. She was taking no medication and had no other remarkable medical records.

At this second presentation, a complete blood count and biochemical study were requested, revealing leukocytosis with neutrophilia, marked thrombocytopenia, increased creatinine, bilirubin and aminotransferases (Table1). The patient was afebrile. An abdominal echography was performed where normal size and morphology of liver, biliary ducts, gallbladder and kidneys were observed. She was admitted at Nephrology Department and kept under observation with a diagnostic workup of acute kidney injury.

On the following day, leukocyte count almost normalized, but a marked decrease on platelet count was seen. Creatinine increased to 7.87 mg/dL, haptoglobin was undetectable and LDH activity was 5,340 U/L. Four to five schistocytes/field were observed in peripheral blood smear. The patient remained anuric since the day of admission. All these findings prompted a work-up for thrombotic microangiopathy (TMA).

DISCUSSION

TMA is a set of pathologies characterized by microvasculature thrombosis and organic dysfunction, originated by different etiologies that can be categorized as congenital or acquired.

The endothelium is injured and the release of von Willebrand Factor (vWF) induces thrombogenesis. Thrombi usually occlude small-caliber blood

Table 1 Evolution of hematological and biochemical parameters

	Ref. interval	Day 1	Day 2	Day 3	Day 5	Day 10	Day 27	After 8 months
Leukocytes, $10^3/\mu L$	3.5 – 11.5	**23.3***	**18.7***	15.3	12.4	14.3	19.3	9.82
Neutrophils, $10^3/\mu L$	2.5 – 11.0	**20.3***	**15.9***	**12.1***	8.04	5.89	10	4.71
Erythrocytes, $10^6/\mu L$	4.00 – 6.00	4.82	4.34	**3.92***	**3.15***	**2.65***	4.46	4.63
Platelets, $10^3/\mu L$	120 - 400	**32.8***	**10.9***	**11.4***	**20.0***	122	179	292
Creatinine, mg/dL	0.57 – 1.11	**5.99***	**7.87***	**9.46***	**7.58***	**9.53***	**1.25***	0.74
Total bilirubin, mg/dL	0.2 – 1.2	**2.9***	**3.4***	**2.4***	**2.1***	0.3	-	-
Direct bilirubin, mg/dL	0.0 – 0.5	**1.3***	-	-	**0.7***	-	-	-
ALT, U/L	0 - 55	**443***	**325***	**121***	-	37	-	-
GGT, U/L	9 - 36	**204***	**159***	**66***	-	**62***	-	-
Haptoglobin, mg/dL	14 - 258	-	**<8***	-	**<8***	-	220	-

CRP, mg/dL	0.0 – 0.5	-	**17.98***	-	-	2.64	0.13	-
LDH, U/L	125 - 220	-	**5,340***	**4,027***	**1,718***	**463***	-	-
Procalcitonin, ng/mL	0.0 – 0.06	-	**> 100***	-	**37.41***	-	-	-

Abbreviations: ALT (alanine aminotransferase); GGT (gamma-glutamyltransferase); HAPT (haptoglobin); CRP (C-reactive protein); LDH (lactate dehydrogenase).
** Values out of the reference interval.*

vessels, especially in the kidney. Obstruction in other organs' vessels may also occur, such as brain, heart, gut, pancreas and lung, hence the observation of common extra-renal symptoms. Blood pressure is usually elevated in patients suffering from TMA, however the one here presented always had values within reference interval. Erythrocyte fragmentation takes place due to friction with the thrombi, thus generating a non-immune hemolytic anemia.

The consensus recommendations in 2016 [1] suggest that TMA must be considered in any patient presenting with microangiopathic hemolytic anemia, thrombocytopenia, schistocytes in peripheral blood (more than one percent) and biochemical signs of non-immune hemolysis (elevated LDH, indirect bilirubin and low haptoglobin levels, with negative Coombs assay). The presence of schistocytes only could be enough in case of clinical evidence.

Procalcitonin, a sepsis biomarker, was greatly increased in this patient despite having negative blood culture results, in accordance with the fact that 40-60% of patients with sepsis may yield a negative blood culture [2]. On day two, intravenous ciprofloxacin was started since enterotoxigenic *Escherichia coli* infection was suspected, however no clinical response was observed and the patient did get worse.

According to etiology, TMA can be classified in: (1) thrombotic thrombocytopenic purpura (TTP), caused by a decreased activity (lower than ten percent) of ADAMTS-13 (a disintegrin- like and metalloprotease with thrombospondin type one motif number 13), which can be of genetic or immune source (antibodies developed after treatment with ticlopidine or clopidogrel); (2) hemolytic uremic syndrome (HUS), as a result of bacterial infections such as the shiga toxin-producing *E coli* or *Streptococcus pneumoniae* (via neuraminidase); (3) atypical HUS (aHUS), associated with genetic or immune complement system alterations (mutations in MCP, CFH, THBD, CFB and C3; antibodies against CFH); and (4) secondary TMA (Table 2) [3].

The best way to start the differential diagnosis is to assess ADAMTS-13 activity. When higher than ten percent, TTP may be ruled out. The patient had an ADAMTS-13 activity of 82%. The second step would be testing for STEC or STEC-like infections. Stool and blood cultures were negative. Urine culture yielded 16,000 cfu/mL of *E coli.*

Although more commonly associated with intestinal STEC infections, HUS can also be induced by urinary tract STEC infections, however this isolated *E coli* strain was not tested for shiga toxin production [4]. Viral serologies (HIV, HAV, HBV, HCV, CMV, EBV and influenza virus) and assays for fecal *E coli* (ECEH, ECEP, ECET, ECEA), *S pneumoniae* and other bacteria were all negative. Bearing in mind these results, an infectious cause was not very likely.

Immunoglobulins were within the reference intervals. The complement study on day two displayed a slight increase in C3 and C4 without clinical relevance.

Rheumatoid factor, ANA, ANCA and anti-glomerular basement membrane were negative. Thus, an autoimmune disease was discarded. Direct and indirect Coombs tests were negative, hence ruling out an autoimmune hemolytic anemia. Pregnancy test was negative.

Methylmalonic aciduria with homocystinuria is produced by a mutation in the CblC gene, due to a deficiency in methylcobalamin and adenosylcobalamin associated with HUS. Although more commonly seen in neonates, two different cases have been reported in adults [5,6].

Table 2 Causes of secondary thrombotic microangiopathy

Causes of secondary TMA	
Pregnancy	HELLP syndrome
	Postpartum
Systemic diseases	Systemic lupus erythematosus
	Antiphospholipid syndrome
	Scleroderma
	Vasculitis
Treatments	C mitomycin, quinine, gemcitabine, cisplatin, ionizing radiation, interferon, VEGF and tyrosine kinase inhibitors (sunitinib, imatinib and dasatinib), ticlopidine, clopidogrel, calcineurin inhibitors (cyclosporine, tacrolimus), sirolimus, valaciclovir, oral contraceptives, etc.
Others	HIV infection
	Glomerulopathy
	Malignant arterial hypertension
	H1N1 infection (influenza A)
	Neoplasia
	Methylmalonic aciduria with homocystinuria
Solid organ or bone marrow transplantation	

Quantification of folate, vitamin B12 and homocysteine could not be performed, as all blood samples were significantly hemolyzed.

Once discarded all other causes in the differential diagnosis, aHUS was suspected. aHUS has a prevalence of one to two cases per million in the USA and 0.11 cases per million in Europe. In children, no gender-dependent incidence has been described, while in adults it is more commonly seen in women. aHUS may emerge at any age, being more frequent in childhood [9].

A screening for possible complement alternative pathway regulatory protein alterations was performed (suspecting of aHUS), including serum alternative pathway H factor (CHF), MCP (Membrane Cofactor Protein; CD46) and I factor concentrations; antibodies anti-H factor; CHF functional alteration assay; and a Western Blot of HF and FHRs. A comprehensive genetic study was also performed, assessing pathogenic variants in the following genes: CFH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, C3, CFI, MCP, CFB, THBD, DGKE, CFP and ADAMTS-13; none of them being found. Heterozygotic change in MCP (CD46) exon 6 (c.686>A, p.Arg229Gln, rs201380032) was detected as variant of unknown significance.

CD46 flow cytometry can be used to assess for genotype/phenotype correlation in unclear cases. Further testing of CFH (H3) risk haplotype polymorphism revealed a deletion in CFHR3-CFHR1 in heterozygosis as well, known to be a common polymorphism in Spanish population, only relevant in homozygosis [7,8]. Biochemical and immunological studies of the complement did not demonstrate any abnormalities.

Genetic variant effect prediction algorithms are used to determine the likely consequences of amino acid substitutions on protein function. The genetic variants prediction study indicated a possible benign effect on the functionality of the protein, as stated by the reference operator laboratory. Furthermore, MCP levels in peripheral blood leukocytes were optimal. The MCP variant detected is not pathogenic and thus not the causal agent of the disease. A few mutations of alternative complement pathway regulatory proteins were described that relate to this syndrome. However, those would only explain 60% of aHUS cases. Some polymorphisms predispose to the development of aHUS when other environmental factors are present.

After five sessions of plasmapheresis and methylprednisolone administration, no response to treatment was observed, so therapy with eculizumab [10] was started on day six. Eculizumab treatment must be initiated only after having confirmed *N meningitidis* vaccination, as the treatment increases the risk of infection by this microorganism due to its mechanism of action (C5 binding, precluding its cleavage into the effector molecules). If the patient is not vaccinated, vaccine must be applied at least 14 days prior to eculizumab initiation. If eculizumab treatment cannot be delayed, appropriate antibiotic prophylaxis must be added since the moment of the vaccination for 14 days. Simultaneous ceftriaxone prophylaxis was set. 48 hours after the first dose of eculizumab, the platelet count increased and LDH activity decreased.

By the day of the medical discharge, creatinine was almost normal. The patient was kept under eculizumab treatment every 14 days, having totally restored her kidney function. After 13 months of treatment and no relapse or complication, eculizumab suspension was decided by the Nephrologist. The patient has not suffered any relapse three months afterwards.

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Manchineel apple of death

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LETTER TO THE EDITOR

Clinical chemistry and analytical toxicology are spiritually intertwined. Interwoven with the blood specimens received in the lab are people's stories, sometimes more commonplace, other times more colourful... which led them there. Usually... seeking refuge under a certain tree during a storm is not one of the stories that bring a person to medical attention.

It is well known to all that in the Bible, Eve offered Adam the poisoned apple. Snow White was offered the poisoned apple which was to send her to sleeping oblivion. The saying goes that an apple a day keeps the doctor away, but consuming numerous crunched apple seeds which containing amygdalin can cause toxicity. Also in the intricacies of nature there exists a fruit which looks a bit like a green small apple and is instead very toxic. This short review delves into how the Manchineel tree has mastered its own art of toxicology.

Trees are generally considered innocuous, however this is not universally so for all trees. We walk or drive past trees on a day to day basis. Nobody hardly ever thinks twice about this.

A glamorous evergreen tree found mostly in tropical regions called the Manchineel tree or *Hippomane mancinella* is a case in point. It is considered the most toxic tree on earth and to that effect added to the 2011 Guinness book of records. Effects may be both dangerous as they could potentially be deadly. Eating that manchineel 'beach apple' can turn out to be quite a toxic unpleasant experience (1).

Consultant radiologist Dr. Nicola Strickland inadvertently tasted the fruit and her symptoms slowly subsided over 8 hours. The taste is initially sweet but the toxic effects soon start kicking in.

Christopher Columbus referred to it as 'manzanilla de la muerte' or the little apple of death.

Even just standing under this tree in the rain can be harmful. Reports of students who took shelter during a storm under this tree gave rise to concurrent dermatitis and ophthalmitis (2). Burning the wood would also result in toxic smoke.

The beach trees exude toxic milky sap with caustic effects. Its latex contains not just skin irritants but also co-carcinogens and cryptic co-carcinogens (3). The sap has been used on poisoned arrows by the Caribs.

Its toxic principles have been long known (4-8). Warning signs and red rings are now being used to alert unwary visitors.

There have been case reports in the literature of manchineel poisoning (9). In this day and age there even is a video testimony on YouTube of an individual allegedly poisoned by manchineel.

Irritant dermatitis is known to occur (10). Contact dermatitis occurs with strong burning and itching sensations with later erythema and bullae formation (11). The characteristic skin reactions have also been referred to as manchineel dermatitis (12). A possible link was made between bradyarrhythmias and Manchineel poisoning (13). Manchineel keratoconjunctivitis and ocular burns have been described (14, 15).

In 1953 an article on accidents due to Manchineel in the Antilles was published in the French journal 'Bulletin de la Societe de pathologie exotique et de ses filiales' (16).

In 2019, a retrospective case series report encompassing 97 patients which had been alerted to French Poison Control Centers over a specific time period was published. Gastrointestinal and oropharyngeal disturbances have been described. They also mention the potential for bradycardia and hypotension. If consumption occurred in larger quantities symptoms may be more severe with haemodynamic disturbance and more severe oropharyngeal injuries (17).

It is claimed that extracts have been used in folk herbal remedies in attempts to treat elephantiasis.

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