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Website: www.isres.org
Contact: isresoffice@gmail.com

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Rovibrationally and Time-Resolved Radiative Lifetime and Collisional Cross Section Measurements of the $6^1\Sigma^+_g$(v=6, J=31) State of Molecular Sodium

Dinesh WAGLE
Miami University

Lok Raj PANT
Miami University

Joseph CONVERSE
Miami University

Scott WENNER
Miami University

Michael SAARANEN
Miami University

S. Burcin BAYRAM
Miami University

Abstract: In recent years, cold and ultracold alkali diatomic molecules have been at the forefront of quantum chemistry and manybody physics. Thus, there has been an increased interest in the determination of the radiative lifetimes for accurate knowledge of the transition dipole matrix elements of these molecules. In this work, we report on radiative lifetime measurements of the sodium diatomic molecule of the $6^1\Sigma^+_g$ (v=6,J=31) and $6^1\Sigma^+_g$ (v=8,J=31) molecular levels by a time-resolved laser spectroscopy technique. The ion-pair character of these potential energies makes their lifetimes interesting because of the unusual behavior of their transition dipole moments. The excitation to the Na_2 $6^1\Sigma^+_g$(3s+5s) electronic state was done using two synchronized pulsed lasers, directed to the sodium heatpipe oven, via $X^1\Sigma^+_g$ (v=0,31) → $A^1\Sigma^+_g$ (9,30) → $6^1\Sigma^+_g$ (8,31) and $X^1\Sigma^+_g$ (v=0,31) → $A^1\Sigma^+_g$ (7,30) → $6^1\Sigma^+_g$ (6,31) transition paths. Disperse molecular fluorescence decay time as a function of argon buffer gas was recorded using a time-correlated photon counting technique. Radiative lifetime was measured and collisional cross section between the excited sodium molecules and ground state argon atoms was extracted.

Keywords: Molecular physics, Spectroscopy, Lifetimes

Introduction

Alkali diatomic molecules have been compelling target for testing fundamental laws of nature and probing the new states of quantum matter to improve precision measurements of fundamental constants, and in the development of quantum information storage and molecule lasers [1-8]. Unlike other alkali dimers, sodium dimer has well separated rovibrational levels, thus it is relatively easier to optically probe the excited electronic states using visible laser light. Since the separation between the vibrational and rotational quantum levels of the sodium dimer are approximately 150 cm^{-1} and 10 cm^{-1} (1 cm^{-1} ≈ 30 GHz) respectively, the pulsed dye lasers are suitable for optical transitions. The study of potential energy curves for the ground and excited states of alkali molecule has always been the matter of interest in atomic, molecular and optical physics area [9,10]. The $6^1\Sigma^+_g$

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(3s+5s) state of Na_2 is of special importance because of the presence of a double well [11], with the secondary well locating at large internuclear distance, as shown in Fig. 1.

In our earlier work, we have reported the radiative lifetime measurement of the $6^1\Sigma^+_g$ (v=7,J=31) state and the calculations of radiative lifetimes in various rovibrational levels of the $6^1\Sigma^+_g$ (3s+5s) state. Our previous calculations revealed a large difference of about a factor of three between the $6^1\Sigma^+_g$ (v=40,J=1) and $6^1\Sigma^+_g$ (v=40,J=31) states due to the wavefunction alternating between having predominantly inner and outer well amplitude. In this work, we performed radiative lifetime measurements of the $6^1\Sigma^+_g$(v=6,J=31) and $6^1\Sigma^+_g$ (v=8,J=31) states of molecular sodium and extracted collisional cross sections. No experimental lifetime and collisional cross section data for these rovibrational levels exist in the literature.

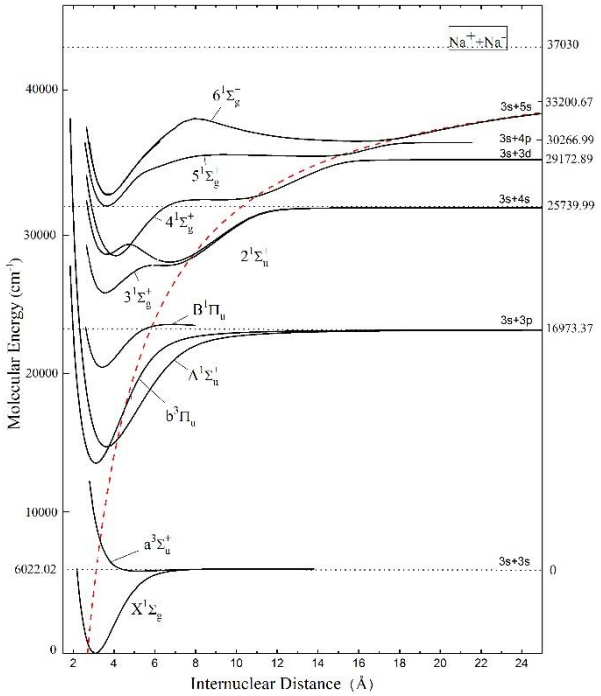

Figure 1. Partial potential energy curves of sodium molecule. The red dotted line represents the ion pair Na^+ + Na^- Coulomb interaction.

Experimental Methods

The experimental setup is shown in Fig. 2. The details of the setup were explained in our previous work [12], hence it will not be presented here. We used a four-armed heatpipe oven to heat sodium metal at desired temperature and pressure. The population of molecules in various rovibrational sates depends on temperature of the oven. The thermal population distribution in various rotational, $(2J+1)e^{-\frac{F(J)}{kT}}$, and vibrational, $e^{-\frac{G(v)}{kT}}$, quantum numbers in the $X^1\Sigma^+_g$ state between temperatures 300°C and 450°C are shown in Fig. 3. Here, F(J) and G(v) are the term values in terms of wavenumber, cm^{-1}. If the transition is initiated from the ground state having higher thermal population distribution of molecules, it is relatively easier to reach the desired rovibrational level (v,J). Two tunable pulsed dye lasers, pumped by a Nd:YAG laser operating in the second harmonics (532 nm) with a pulse repetition rate of 20 Hz, were used for the two-step excitation of sodium molecules into the $6^1\Sigma^+_g$ state. After the satisfaction of a double resonance condition a spectrometer-CCD/PMT system was used to identify the peaks and make lifetime measurements. The dispersed molecular fluorescence was measured at right angles to the propagation direction of the lasers and the signal was collected through a photomultiplier (PMT)-multichannel scalar (MCS) photon counting set-up.

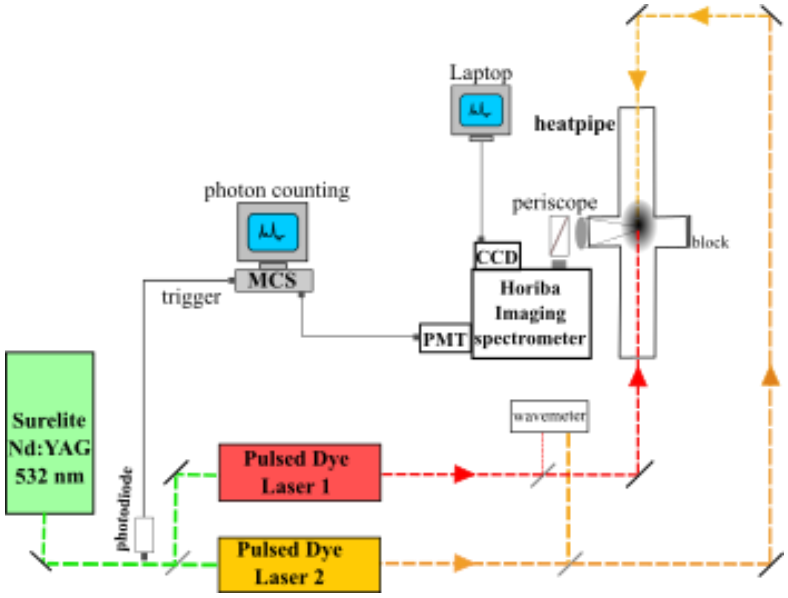

Figure 2. Experimental setup

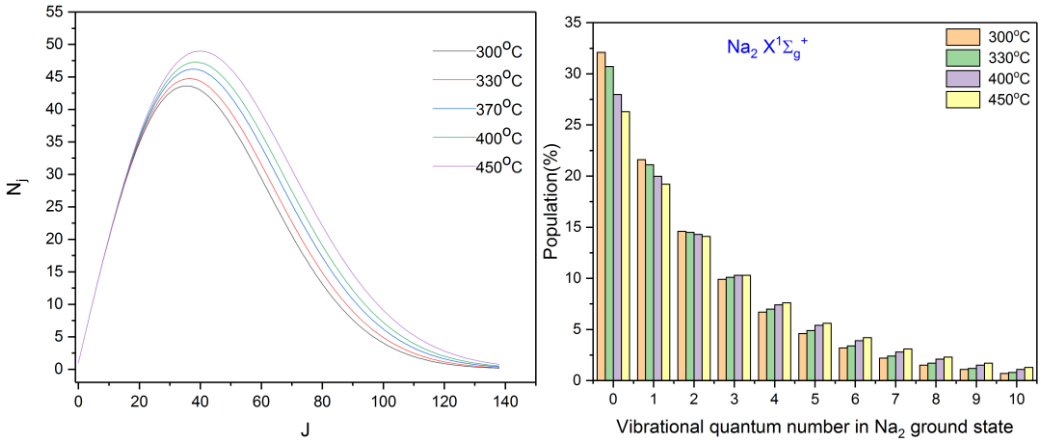

Figure 3. (Left) Rotational population distribution (N_j) as a function of rotational quantum number (J) in the ground state (B = 0.154 cm^{-1}), (Right) Relative thermal population as a function of vibrational quantum number

Lifetime Measurements of the $6^1\Sigma^+_g$ (v=6, J=31) and $6^1\Sigma^+_g$ (v=8, J=31) states of Na_2

The spectral peaks for the double resonance transition were identified using the Fortran LEVEL 8.0 program. In this program experimental potential energy curves are used to determine the Frank-Condon factors (FCFs), Einstein coefficients, energies of rovibrational levels, and dipole moment matrix elements. FCFs represent the probability of transition between two rovibrational states, and the intensity of peaks is proportional to FCF. The transition to excite the $6^1\Sigma^+_g$ (8,31) state is shown in Fig. 4. Due to high FCFs, the rovibrational levels (8,31) and (6,31) of the $6^1\Sigma^+_g$ state of Na_2 have been populated via the following resonant transitions:

$X^1\Sigma^+_g$ (v=0,31) → $A^1\Sigma^+_g$ (9,30) → $6^1\Sigma^+_g$ (8,31) and $X^1\Sigma^+_g$ (v=0,31) → $A^1\Sigma^+_g$ (8,30) → $6^1\Sigma^+_g$ (7,31).
Laser L_1 (1.8 mW power) drives the $X^1\Sigma^+_g$ (v=0,31) → $A^1\Sigma^+_g$ (9,30) transition at 639.84 nm, while laser L_2 (2.8 mW power) drives $A^1\Sigma^+_g$ (9,30) → $6^1\Sigma^+_g$ (8,31) transition at 561.80 nm. Similarly, for the excitation to the $6^1\Sigma^+_g$ (6,31) state, L_1 drives the $X^1\Sigma^+_g$ (v=0,31) → $A^1\Sigma^+_g$ (7,30) transition at 650 nm, while laser L_2 drives $A^1\Sigma^+_g$ (7,30) → $6^1\Sigma^+_g$ (6,31) transition at 561.80 nm. Both lasers are synchronized and the overlap time of the pulses are about 3 ns. After the two-step excitation a rovibrationally resolved molecular fluorescence spectrum from the $6^1\Sigma^+_g$ state was acquired. Figures 5 and 6 show fluorescence spectra from the $6^1\Sigma^+_g$ (6,31) and $6^1\Sigma^+_g$ (8,31) states. The double-resonance condition was confirmed by separately blocking the lasers. The spectrum shows P- and R- branches and the wavelengths for the peaks were identified comparing them with the theoretical values, obtained using LEVEL 8.0 Fortran program. High intensity of the spectral peaks suggest that the FCF of the

transition is higher. The FCF of the transitions at various wavelengths from the $6^1\Sigma^+_g$ (8,31) and $6^1\Sigma^+_g$ (6,31) state. The separation between the P- and R- branches of the doublets is approximately 13.40 cm^{-1}.

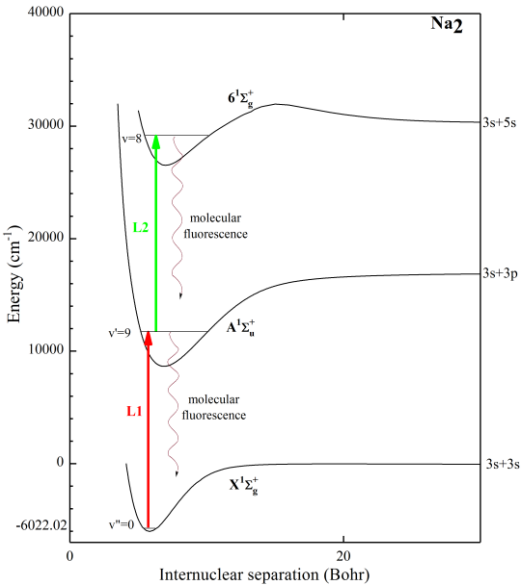

Figure 4. Partial energy curves showing excitation scheme for the $6^1\Sigma^+_g$ (8,31) state

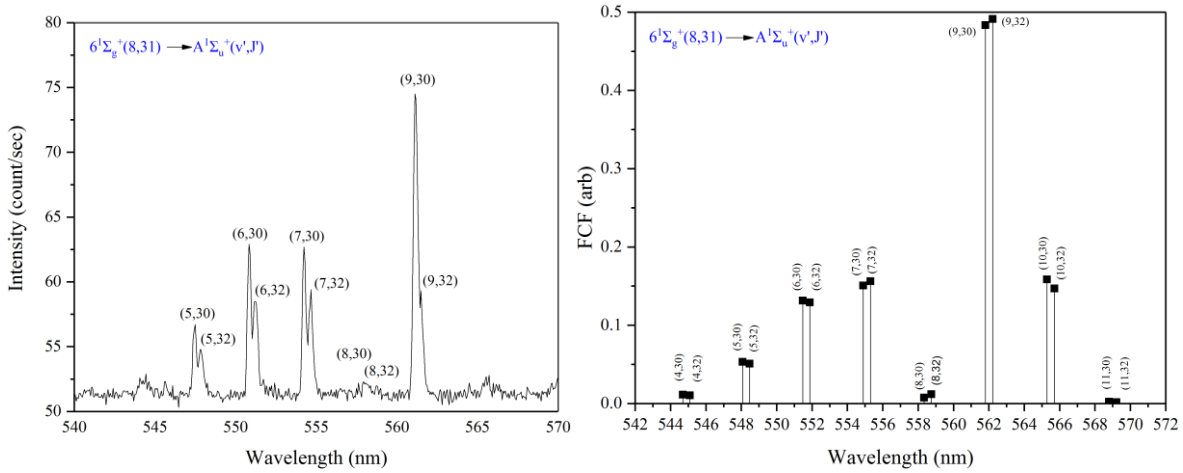

Figure 5. (Left) Molecular fluorescence, (Right) intensity spectrum showing relative Franck-Condon factors from the $6^1\Sigma^+_g$ (8,31) state to the $A^1\Sigma^+_g$ state

The typical decay signal of the $6^1\Sigma^+_g$ state as a function of time using time-correlated single photon counting method via multichannel scaler is shown in Fig. 7. The data shows the exponential drop of counts as a function of time where the red line is the convolution fit of a Gaussian with an exponential curve function. The effective lifetimes (τ_e) were extracted from the convolution fit using OriginLab 2017. Each data is the average of five measurements. Each measurement is the accumulation of 20000 scans. This process was repeated at various argon pressures. The inverse of the measured lifetimes were plotted against the corresponding pressures. This gives a linear relationship which is called Stern-Volmer plot as shown in Figure 8. The reciprocal of the intercept of the linear fit provides radiative lifetime. The radiative lifetime of the $6^1\Sigma^+_g$ (8,31) state is found to be 15± 1 ns at 300°C and that of the $6^1\Sigma^+_g$ (6,31) state is 13±1 ns at 330°C.

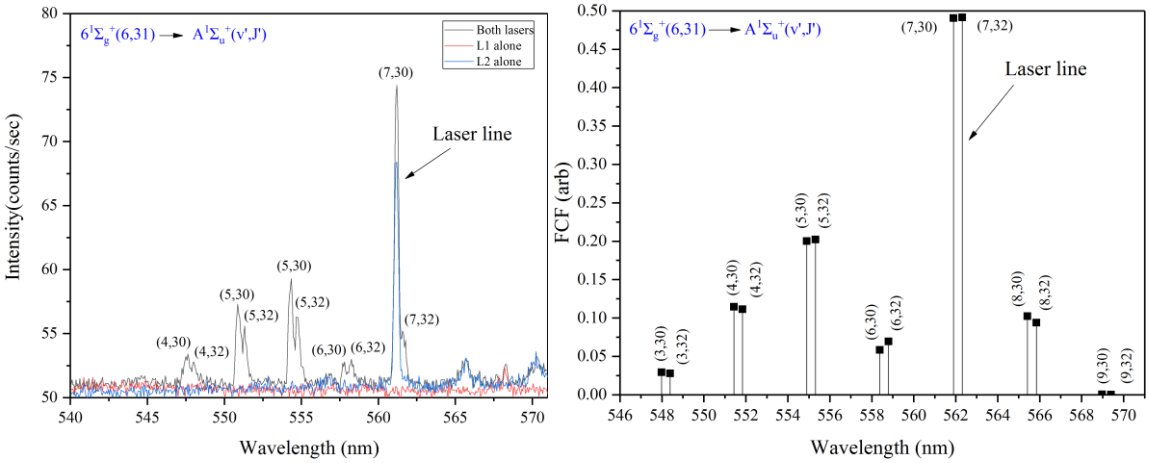

Figure 6. (Left) Molecular fluorescence, (Right) intensity spectrum showing relative Franck-Condon factors from the $6^1\Sigma^+_g$ (6,31) state to the $A^1\Sigma^+_g$ state

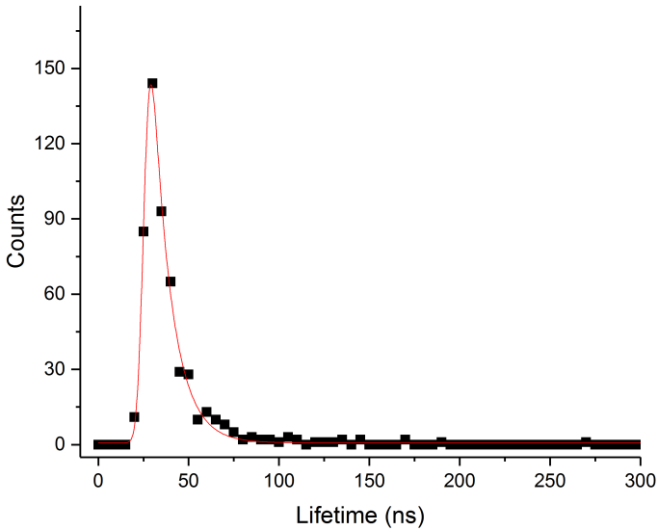

Figure 7. A typical lifetime measurement: the red line is a convolution fit of a Gaussian with an exponential curve function to the data.

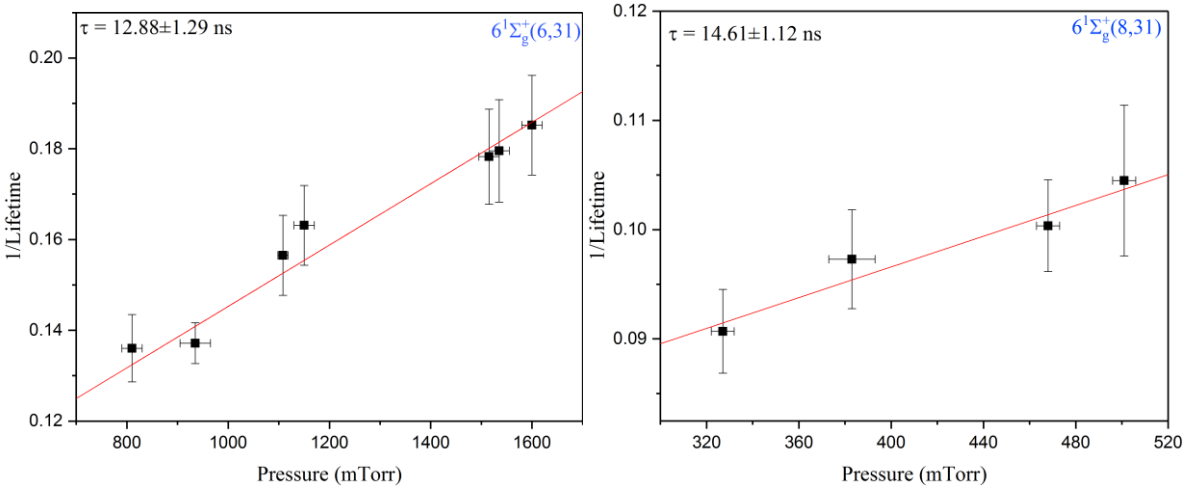

Figure 8. Stern-Volmer plots showing the variation in the inverse lifetimes as a function of Ar pressures

Collisional Cross Section

In the experiment, we used argon as a buffer gas and thus there are collisions among the sodium dimers, sodium atoms and argon atoms. As the argon pressure increases, the excited state sodium dimers may decay to other states by collisions. The collisional cross section of the excited state can be determined from the slope of the Stern-Volmer plot.

The effective lifetime (τ_e), radiative lifetime (τ_r) and non-radiative lifetime (τ_{nr}) are related by the Stern-Volmer relationship [13] which can be written as

$$\frac{1}{\tau_e} = \frac{1}{\tau_r} + \frac{1}{\tau_{nr}}$$

where $\frac{1}{\tau_{nr}} = N_p \bar{v}\, \sigma$. Here, N_p is the number density of the buffer gas, $\bar{v}$ is the average velocity of the colliding Na_2* - Ar atoms over the Maxwell-Boltzmann distribution of relative velocities at the heatpipe oven temperature, and σ is the collisional cross section between the excited dimer and Ar atoms. The number density of the buffer gas is determined by measuring the pressure at room temperature and applying the ideal gas law:

$$N_p = \frac{n}{V} = \frac{P}{\mathrm{RT}}$$

Also, average velocity is calculated using Maxwell-Boltzmann statistics:

$$\sqrt{\frac{8k_B T}{\pi\mu}}$$

Thus, the Stern-Volmer relationship reduces to the following:

$$\frac{1}{\tau_e} = \frac{1}{\tau_r} + \frac{\bar{v}}{\mathrm{k}_B T}\sigma P$$

The slope of the equation ($\frac{\bar{v}}{k_B T}\sigma$) yields the collisional cross section while the intercept (1/ τ_e) at zero pressure yields the radiative lifetime.

The reciprocal of the intercept of the linear fit is the radiative lifetime. The radiative lifetime of the $6^1\Sigma^+_g$ (8,31) is measured to be 15±1 ns at 300°C and that of the $6^1\Sigma^+_g$ (6,31) state is 13±1 ns at 330°C. We measured the collisional cross section of the Na_2 $6^1\Sigma^+_g$ (8,31) state to be 555±100 Å^2 at 300°C and the $6^1\Sigma^+_g$ (6,31) state to be 546±56 Å^2 at 300°C.

Results and Discussion

We employed the technique of time-resolved spectroscopy to measure for the first the radiative lifetimes of the excited electronic states, $6^1\Sigma^+_g$ (8,31) and $6^1\Sigma^+_g$ (6,31) of sodium molecules. For the $6^1\Sigma^+_g$ state, the observed fluorescence is the result of double resonance molecular emission from the target rovibrational level. We have confirmed the peaks from the fluorescence emission with the results calculated using the LEVEL 8.0 Fortran program. Using a photon counting technique, we measured the effective lifetimes at various argon pressures and the radiative lifetime was extracted from the extrapolation of the linear fit to the zero pressure limit using a Stern-Volmer plot. Also, this plot is used to extract the collisional cross section at the operating temperature of the heatpipe. The obtained data are presented in Table 1. The measured radiative lifetimes of Na_2 $6^1\Sigma^+_g$ (v=8,J=31) and $6^1\Sigma^+_g$ (v=6,J=31) (even vibrational states) are in agreement within the error limits but both are smaller compared to the $6^1\Sigma^+_g$ (v=7,J=31) (odd vibrational state) [4]. Radiative lifetime oscillations in the Na_2 $4^1\Sigma^+_g$ state were reported [6].

Table 1. Tabulated data for radiative lifetimes (τ), temperatures (T), and collisional cross sections (σ)

State	τ (ns)	T (K)	σ (Å^2)	Source
$6^1\Sigma^+_g$ (8,31)	15±1	573	555 (100)	This work
$6^1\Sigma^+_g$ (7,31)	40±2	563	573 (35)*	Ref.[4]
$6^1\Sigma^+_g$ (6,31)	13±1	603	546 (56)	This work

*This work, which is calculated using experimental results from previous work [4]

It is possible, in principle, for the intermediate state molecules to be excited to a higher nearby rovibrational levels by collisions. The collisional kinetic energy depends on the k_BT, which is on the order of few hundred wavenumber. The separation between the rotational levels is about 13 cm^{-1} and the vibrational levels are 150 cm^{-1}. The shorter lifetimes for the v=6 and 8 states could be the perturbation in the intermediate A state due to the ion-pair state of sodium dimers. It is also possible for the molecules in the intermediate A state to be excited to a higher rovibrational levels by collisions. The shorter lifetimes maybe related to the excited state Na_2 collisions with the ground state iodine molecule or due to weak perturbations in the intermediate A state. Our results showed that the collisional cross sections for the excited $6^1\Sigma^+_g$ in the v= 6, 7, and 8 states are in excellent agreement within the error limits.

Conclusion

We employed the technique of time-resolved spectroscopy to measure the radiative lifetimes and collisional cross sections of the highly excited electronic states, $6^1\Sigma^+_g$ (8, 31) and $6^1\Sigma^+_g$ (6, 31), of sodium molecules for the first time. For the $6^1\Sigma^+_g$ state, the observed fluorescence is the result of double resonance molecular emission from the target rovibrational level. Using a photon counting technique, we measured the effective lifetimes at various argon pressures and the radiative lifetime was extracted by extrapolating the linear fit to the zero pressure limit of the Stern-Volmer plot. Further, a Stern-Volmer relationship is used to extract the collisional cross sections at the operating temperature of the heatpipe. The measured collisional cross sections for the v=6, 7, and 8 quantum states are found to be in excellent agreement within the error limits. The measured radiative lifetimes of Na_2 $6^1\Sigma^+_g$ (v=8,J=31) and $6^1\Sigma^+_g$ (v=6,J=31) (even vibrational states) are in excellent agreement with each other but both are smaller compared to the $6^1\Sigma^+_g$ (v=7,J=31) (odd vibrational state) [12]. Similar lifetime variations in the Na_2 $4^1\Sigma^+_g$ state were reported [14]. Hence, considerable differences in lifetimes for the v=6, 8 quantum numbers will be further investigated.

Recommendations

Prof. Noureddine Melikechi (atomic, molecular physics); E:mail: Noureddine_Melikechi@uml.edu
Prof. Mark Havey (atomic, molecular physics); E:mail: mhavey@odu.edu
Prof. Marjatta Lyyra (molecular physics); E:mail: lyyra@temple.edu
Prof. Gonul Basar (atomic spectroscopy); E-mail: gbasar@istanbul.edu.tr

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Author Information

Dinesh Wagle
Miami University
500 E. Street, 219 Kreger Hall,
Oxford, OH, 45056, USA
Contact E-mail: *wagled@miamioh.edu*

Lok Raj Pant
Miami University
500 E. Street, 219 Kreger Hall,
Oxford, OH, 45056, USA

Joseph Converse
Miami University
500 E. Street, 219 Kreger Hall,
Oxford, OH, 45056, USA

Scott Wenner
Miami University
500 E. Street, 219 Kreger Hall,
Oxford, OH, 45056, USA

Michael Saaranen
Miami University
500 E. Street, 219 Kreger Hall,
Oxford, OH, 45056, USA

Burcin Bayram
Miami University
500 E. Street, 219 Kreger Hall,
Oxford, OH, 45056, USA

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X-Chromosome and Abnormalities

Mehmet OZASLAN
Gaziantep University

Sibel BAYIL OGUZKAN
Gaziantep University

Abstract: This research investigates the function of the X chromosome and its different abnormalities on humans, including data from comparative genome analysis of other organisms. The X chromosome has many fatures that are unique in the human genome. Females inherit an X chromosome. Gene expression on one of the female X chromosomes is silenced early in development by the process of X-chromosome inactivation (XCI), and this chromosome remains inactive in somatic tissues thereafter. In the female germ line, the inactive chromosome is reactivated and undergoes meitotic recombination with the second X chromosome. The male X chromosome fails to recombine along virtually its entire length during meiosis: instead, recombination is restricted to short regions at the tips of the X chromosome arms that recombine with equivalent segments on the Y chromosome. Genes inside these regions are shared between the sex chromosomes, and their behaviour is therefore described as 'pseudoautosomal'. Genes outside these regions of the X chromosome are strictly X-linked, and the vast majority are present in a single copy in the male genome. The unique properties of the X chromosome are a consequence of the evolution of sex chromosomes in mammals. The sex chromosomes have evolved from a pair of autosomes within the last 300 million years. In the process, the original, functional element have been conserved on the X chromosome, but the Y chromosome has lost almost all traces of the ancestral autosome, including the genes that were once shared with the X chromosome. The hemizygosity of males for almost all X chromosome genes exposes recessive phenotypes, yhus accounting for the large number of diseases that have been associated with the X chromosome.The biological consequences of the sex chromosome evolution account fort the intense interest in the human X chromosome in recent decades. However, evolutionary processes are likely to have shaped the behaviour and structure of the X chromosome in many the ways, influencing features such as repeat content, mutation rate, gene content and haplotype structure. The availability of the finished sequence of the human X chromosome, described here, now allows us to explore its evolution and unique properties at a new level.

Keywords: X chromosome, XCI, Y chromosom

Introduction

Identifying gens on each chromosome in an active area of genetic research. Buceuse researchers use different approaches to predict the number of genes on each chromosome, the estimated number of genes varies The X chromosomelikely contains 800 to 900 genes that provide instructions for making proteins. These proteins perform a variety of different roles in the body. The X chromosome in humans spans more than 153 million base pairs (the building material of DNA). It represents about 800 protein-coding genes compared to the Y chromosome containing about 70 genes, out of 20,000-25,000 total genes in the human genome. Each person usually has one pair of sex chromosomes in each cell. Females have two X chromosomes, whereas males have one X an done Y chromosome. Both males and females retain one of their mother's X chromosomes, and females retain their second X chromosome from their father. Since the father retains his X chromosome from his mother, a human female has one X chromosome from her paternal grandmother (father's side), an one X chromosome from her mother (1). This inheritance pattern follows the Fibonaccci numbers at a given ancestral depth. Genetic disorders that are due to mutations in genes on the X chromosome are decribed as **X linked.** The X chromosome carries hundreds of genes but few, if any, of these have anything to do directly with sex

determination. Early in embryonic development in females, one of the two X chromosome is randomly and permanently inactivated in nearly all somatic cells (cells other than egg and sperm cells). This phenomenon is caled X-inactivation or iyonization, and creates a Barr body. If X-inactivation in the somatic cell meant a complete de-functionalizing of one of the X- chromosomes, it would ensure that females, like males, had only one fuctional copy of the X chromosome in each somatic cell (2,3). This was preciously assumed to be the case. However, recent research suggests that the Barr body may be more biologically active than was previously supposed. Genetic disorders that arise from missing, additional of malformed copies of the X chromosome are termed numerical disorders. Examples include Klinefelter's syndrome where a male has one or more extra copies; Triple X syndrome, where a female has one extra copy and Turner syndrome where a female has one normal X chromosome an done missing or abnormal one (4).

Structure of X Chromosomes

It is theorized by Ross et al. 2005 and Ohno 1967 that the X chromosome is at least partially derived from the autosomal (non-sex-related) genome of other mammals, evidenced from interspecies genomic sequence elignments. The X chromosome is notably larger and has a more active euchromatin refion than its Y chromosome counterpart. Futher comparison of the X and Y reveal regions of homology between the two.

However, the cerresponding region in the Y appears far shorter and lacks regions that are conserved in the X throughout primate speciens, implying a genetix degeneration for Y in that region. Because males have only one X chromosome, they are more likely to have an X chromosome-related disease.

It is estimated that about 10% of the genes encoded by the X chromosome are associated with a family of ''CT'' genes, so named because they encode for markers found in both tumor cells (in cancer patients) as well as in the human testis (in healthy patients) (5).

Health conditions related to chromosomal changes

The following a few chromosomal conditions are associated with changes in the structure or number of copies of X chromosome

a) 46,XX testicular disorder of sex development

In most individuals with 46,XX testicular disorder of sex development, the condition results from an abnormal Exchange of genetix material between chromosomes (translocation). This Exchange occurs as a random event during the formation of sperm cells in the affected person's father. The translocation affects the gene responsible for development of a fetus into a male (the SRY gene). The SRY gene, which is normally found on the Y chromosome, is misplaced in this disorder, almost always onto an X chromosome. A fetus with an X chromosome that carries the SRY gene will develop as a male despite not having a Y chromosome.

b) Intestinal pseudo-obstruction

Intestinal pseudo-obstruction, a condition characterized by impairment of the muscle contractions that move food through the digestive tract (peristalsis), can be caused by genetic changes within the X chromosome. Some individuals with intestinal pseudo-obstruction have mutations, duplications, or deletions of genetic material in the X chromosome that affect the FLNA gene. Researchers believe that these genetix changes may impair the function of the filaminA protein , causing abromalities in the cytoskeleton of nevre cells (neurons) in the gastrointestinal tract. These abromalities result in impaired peristalsis, which causes abdominal pain and the other gastrointestinal symptoms on intestinal pseudo-obstruction. Deletions or duplications of genetix material that affect the FLNA gene can also include adjacent genes on the X chromosom. Changes in adjacent genes may account for some of the other signs and syptoms, sucg as neurological abromalities and unusual facial features, that ocur in some affected individuals.

c) Klinefelter syndrome

Klinefelter syndrome is caused by the presence of one or more exta copies of the Xchromosome in a male's cells. Exta genetic material from the X chromosome interferes with male sexual development, preventing the testes from functioning normally and reducing the levels of testosterone. A shortage of testosterone can lead to

delayed or incomplete puberty, genital abnormalities, breast enlargement (gynecomastia), reduced facial and body hair, and an inability to have biological children (infertility). Children with Klinefelter syndrome, and a shy and unassuming personality. Typically, people with Klinefelter syndrome have one exta copy of the X chromosome in each cell, for a total of two X chromosome and one Y chromosome (47,XXY). Less commonly, affected individuals may have two or three extra X chromosomes (48,XXXY or 49,XXXXY). As the number of extra sex chromosomes increases, so does the risk of learning problems, intellectual disability, birth defects, and other health issues. Some people witht features of Klinefelter syndrome have the extra X chromosome in only some of their cells; in these individuals, the condition is described as mosaic Klinefelter syndrome (46,XY/47,XXY). Individuals with mosaic Klinefelter syndrome may have milder signs and symptoms, depending on how many cells have an additional X chromosome.

d) Triple X syndrome

Triple X syndorme (also called 47,XXX or trisomy X) results from an exta copy of the X chromosome in each of a female's cells. Females with triple X syndrome have three X chromosomes, for a total of 47 chromosomes per cell. An exta copy of the chromosome is associated with tall stature, learning problems, and other features in some girls and women.
Some females with X syndrome have an extra X chromosome in only some of their cells. This phenomenon is called 46,XXX mosaicism.
Females with more than one extra copy of the C choromosome (48,XXXX or 49,XXXXX) have been identified, but these choromosomal changes are rare. As the number of extra sex choromosomes increases, so does the risk of learning problems, intellectual disability,birth defects,and other health issues.

e) Turner syndrome

Turner syndrome results when one normal X chromosome is present in a female's cells and the order sex choromosome is missing or structurally altered. The missing genetic material affects development before and after birth, leading to short stature, ovarian malfunction, and the other features of Turner syndrome. About half of individuals with Turner syndrome have monosomy X (45, X) , which means each cell in an individual's body has only one copy of the X chromosome instead of the usual two sex chromosomes. Turner syndrome can also ocur if one of the sex choromosomes is partially missing or rearranged rather than completely absent. Some women with Turner syndrome have a chromosomal change in only some of their cells, which is known as mosaicism.

Some cells have usual two sex choromosomes (either two X choromosomes or one X choromosome an done Y chromosome), and other cells have only one copy of the X choromosome. Women with Turner syndrome caused by X choromosome mosaicism (45,X/46, XX or 45 X/46, XY) are said to have mosaic Turner syndrome. Researchers have not determined which genes on the X chromosome are responsible for most of the features of Turner syndrome. They have, however, identified one gene called SHOX that is important for bone development and growth. The SHOX gene is located in the pseudoautosomal regions of the sex xhoromosomes. Missing one copy of this gene likely causes short stature and skeletal abnormalities in women with Turner syndrome (6,7).

Results and Discussion

The X chromosome has many genes related to growth and development. The Y chromosome is smaller and contains fewer genes. Females have two X chromosomes (XX), so when one copy of the gene on one X chromosome changes, the normal gene copy on the other X chromosome is sufficient for normal growth and development, briefly supporting the modified copy. In such a situation, the female individual is healthy but carrier for the disease caused by X. Carrier means that an altered copy of the gene is transported without the patient. In some cases, females may show some signs of the disease. Although scientists have known this phenomenon, called X chromosome inactivation, for more than fifty years, little is still known about how it occurred or how it developed. At the same time, every copy of the X chromosome contains a variety of genes that are not in another. So having two X chromosomes gives females greater genetic diversity than males with one X chromosome. For this reason, ideas have been discussed in recent years as females have a genetic diversity that scientists have just begun to understand. We think that detailed cytogenetic studies should be done on this subject.

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Author Information

Mehmet Ozaslan
Department of Biology, University of Gaziantep, Gaziantep, Turkey
Contact E-mail: *ozaslanmd@gantep.edu.tr*

Sibel Bayil Oguzkan
Department of Medical Services and Techniques, Health Services, University of Gaziantep, Gaziantep, Turkey

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Comparison Effect of Different Drying Methods on Physicochemical Properties and Antioxidant Activity of Pineapple Fruits

Sibel BOLEK
University of Health Sciences

Abstract: Thanks to their high fiber, antioxidant activity, vitamin and mineral content, dried fruits are much healthier and more nutritious snacks than other processed snack foods. However, many research shows that certain compounds may degrade in the drying process, notably antioxidants, so drying method is very important to obtain desirable and healthy dried products. In this study, the comparison of quality characteristics of fluidized bed, microwave and vacuum dried pineapples was investigated. Pineapple slices were dried using fluidized bed dryer at 80 °C for 3 h. Vacuum drying treatments were performed using vacuum oven at 21.5 kPa and 60 °C for 12 h. Drying treatments in a microwave oven were performed at 360 W for 24 min. Color, rehydration capacity, shrinking ratio, antioxidant activity, total phenolic content measurements and sensory analysis were conducted in order to compare the physical, chemical and sensory properties of dried pineapples. Microwave dried samples had the highest L* value, total phenolic content and antioxidant activity compared to other samples ($p < 0.05$). On the other hand, vacuum dried samples took the highest flavor and overall impression values by the panelists ($p < 0.05$). When it was compared to other drying methods used in this study, fluidized bed drying caused the highest decrease in physicochemical, antioxidant activity and of pineapple samples. In the result of this study, it was revealed that being quick, cheap, and practical method, microwave drying has a promising potential for high-quality dried products.

Keywords: Drying, Pineapple, Antioxidant activity, Sensory

Introduction

Drying, one of the oldest food preservation methods, is the simple and practical process of dehydrating foods until there is not enough moisture to support microbial activity. Drying removes the water needed by bacteria, yeasts, and molds need to grow. When they adequately dried and properly stored, dehydrated foods are shelf stable and convenient to store. The drying food preservation method is easy to do, very safe, and can be used for most types of foods (meats, fruits, and vegetables). Many different foods can be prepared by dehydration. Moreover, dried foods are good sources of quick energy and wholesome nutrition. Therefore, dried foods are healthy alternatives for busy executives, hungry backpackers and active women and children, all of whom can benefit from the ease of use and nutritional content of dried foods.

Pineapple (*Ananas comosus L.*) is a member of the botanical family Bromeliaceae. It is rich sources of ascorbic acid, minerals, fibers, and antioxidants. (Ramallo & Mascheroni, 2012). Pineapple fruit is a good way to increase the nutritive value of poor people's diets and to reduce dietary deficiencies (Da Silva et al., 2013). Pineapple fruit is consumed fresh and/or processed as juice or canned. Additionally, drying pineapple slices and rings can be used as an alternative processing technology with the ability to enrich this raw material and expand consumption.

There are many food drying methods including solar drying, freeze drying, vacuum drying, microwave drying, drum drying, spray drying. Each has its own advantages and limitations. The final product obtained from these methods may differ in physical, chemical, and nutritional properties (Caparino et al., 2012). Since the physical and chemical properties of dried foods depend on the drying methods and drying conditions, it is critical to determine the appropriate drying method and drying conditions. Vacuum drying is a process in which materials are dried in a reduced pressure environment, which lowers the heat needed for rapid drying (Parikh, 2015).

Fluidized bed drying allowing uniform drying of food items because heat from the air flow goes directly to the surface. Microwave drying is caused by water vapour pressure differences between interior and surface regions which provide a driving force for moisture transfer (Maskan, 2001). Microwave drying offered an alternative way to improve the quality of dehydrated products.

Only a few studies have been conducted regarding drying of pineapple fruit to extend the shelf life. Therefore, the objective of this study is the comparison of the freeze drying, vacuum drying and microwave drying methods for the processing of pineapple fruit in respect to drying, color, rehydration capacity, shrinking ratio, antioxidant activity, total phenolic content measurements and sensory analysis obtained by the three drying techniques.

Material and Methods

Metarials

In all the experiments in this study, wholly matured and healthy pineapples were used. Fresh fruits (Golden sweet) were purchased from a farmer's market in Bursa, Turkey. The fruits were then stored until analysis at a temperature of 4 ± 0.5°C.

Using a food slicer, the samples were sliced into pieces 3 ± 0.04 mm.

Drying Process

Pineapple slices were dried using fluidized bed dryer at 80 °C for 3 h. Vacuum drying treatments were performed using vacuum oven at 21.5 kPa and 60 °C for 12 h. Drying treatments in a microwave oven were performed at 360 W for 24 min.

Color Analysis

Color values (L*, a* and b*) was measured using a chroma meter (model CR-400, Konica Minolta Inc., Osaka, Japan) equipped with a D65 illuminant and operating with CIE L*a*b* color space. Calibration was performed with the white color calibration tile prior to the color measurements.

Determination of Rehydration Ratio

Rehydration experiments for dried samples at 40°C were carried out in distilled water bath at temperature of 25 and 50°C (±1°C). Approximately 1 g of dried samples was added to 400 mL distilled water, in a 500 mL beaker. The sample was withdrawn from the liquid every 20 min, and excess water was carefully removed by blotting on a tissue paper, before weighing. The rehydration ratio (RR) was calculated by the following equation:

$$RR = Wr/Wd$$

where Wr is the weight of moisture (kg) and Wd is the weight of dry matter (kg).

Determination of Shrinkage Ratio

The shrinkage/volume change of the samples was expressed as a bulk shrinkage ratio of sample volume at any time to initial volume.

Antioxidant Activity Measurements

Antioxidant activity was determined according to method described by Singleton et al. (1999). (DPPH (2,2-diphenyl-1-picrylhydrazyl)-free radical scavenging activity of the pineapple extracts was spectrophotometrically evaluated. To 3.9 mL of a 25 mM methanolic solution of the DPPH radical (Sigma-Aldrich, Germany), 0.1 mL of the appropriately diluted extract was added, and the mixture was vortexed for 15–30 s. The reaction of the mixture was allowed to proceed in the dark at room temperature for 30 min. After this, the absorbance was

measured at 515 nm (UV Lambda 35, Perkin-Elmer Corp., Shelton, CT, USA). The results were expressed as μmol Trolox equivalents (TE)/g d.w. Three sets of each sample were analyzed.

Determination of Total Phenolic Content

Total phenolic content was determined using a slight modification of the procedure described by Cemeroğlu (2009). Briefly, 5-g samples were homogenized by beating in a mortar with 50 mL of 80% methanol for 5 min. The homogenate was boiled for 5 min in a beaker. The extract was filtered by Whatman 4 filter. The residue in the beaker was reboiled for 10 min by adding 50 mL of 80% methanol. The 2 extracts were combined in 100-mL volumetric flasks and allowed to cool. After cooling, the flask was completed with distilled water, 5 mL of the extract was taken into a 50-mL volumetric flask, and 5 mL of distilled water was added to it. Then 0.5 mL of Folin–Ciocalteu reagent was added, and the container was shaken vigorously. After waiting for 3 min, 1 mL of 36% sodium carbonate solution was added. The container was then filled with distilled water and shaken strongly. The container was left in a dark environment for 1 h. The absorbance at 725 nm was measured afterwards.

Sensory Analysis

A panel of 24 judges carried out a sensory evaluation using a hedonic scale (Altuğ-Onoğur and Elmacı, 2011). Appearance, texture, flavor, and overall impression were evaluated. Panelists had experience in evaluating different food products.

Statistical Analysis

The experiment was conducted using a completely randomized design with 3 replications. Data were analyzed using the PROC MIXED procedure of SAS (SAS, 1999).

Results and Discussions

Effects of drying process on color values of pineapple slices are given in Table 1. Color is one of the most important quality attributes of foods because it directly influences consumer acceptability. Drying methods affected the color parameters of the dried pineapple fruits. L* and b* values in all the dried samples decreased compared with the fresh fruit. On the other hand, a* value of the fresh sample significantly ($P < 0.05$) lower than that of any other drying method. The decrease in L* value could be explained by Maillard reactions that contribute to the formation of colored compounds, called melanoidins (Bolek & Ozdemir, 2017). Fluidized bed dried pineapple slices presented the lowest L* value. L *and b* value of dried pineapples slices by vacuum and microwave are not different significantly ($p > 0.05$).

Table 1. Color values of pineapple slices

Drying method	**L***	**a***	**b***
Fresh	70.12 ± 0.45^{a}	0.49 ± 0.02^{c}	41.88 ± 0.12^{a}
Vacuum	56.21 ± 1.10^{b}	11.32 ± 0.58^{b}	37.90 ± 1.15^{b}
Microwave	54.42 ± 1.16^{b}	11.85 ± 0.32^{b}	36.21 ± 1.52^{c}
Fluidized bed	47.20 ± 1.25^{c}	12.89 ± 0.22^{a}	29.57 ± 1.60^{c}

a–c: Means having a different subscript within a column differ ($p < 0.05$).

Effects of drying process on rehydration and shrinkage ratios are given in Table 2. Drying methods affected the rehydration and shrinkage ratio of the dried pineapple fruits significantly ($p<0.05$). Rehydration ratio is very important quality characteristic for drying fruits. It depends on processing conditions, sample preparation, sample composition and extent of the structural and chemical disruption induced by drying (Singh et al., 2006). The rate of water removal influences the extent of shrinkage during air drying of tissue foods. Rehydration

capacity and shrinkage ratio of vacuum dried and microwave dried pineapple slices are higher than fluidized bed dried pineapple slices.

Table 2. Rehydration and Shrinkage Ratio of dried pineapple slices

Drying method	**Rehydration Capacity**	**Shrinkage Ratio**
Vacuum	4.18 ± 0.02^{a}	0.82 ± 0.03^{a}
Microwave	4.10 ± 0.01^{a}	0.76 ± 0.02^{a}
Fluidized bed	3.36 ± 0.02^{b}	0.40 ± 0.03^{a}

a–b: Means having a different subscript within a column differ ($p < 0.05$).

Effects of drying process on antioxidant activity and total phenolic content of pineapple slices are given in Table 3. Drying method affected antioxidant activity and total phenolic contents of pineapple slices significantly ($p< 0.05$). Fluidized bed drying caused a decrease in antioxidant activity and total phenolic content of pineapple slices more than vacuum drying and microwave drying ($p < 0.05$).

Table 3. Antioxidant activity and total phenolic content of pineapple slices

Drying Method	**Antioxidant Activity µmol Trolox/g**	**Total Phenolic Content mg GAE/100 g**
Fresh	14.55 ± 0.09^{a}	468.52^{a}
Vacuum	8.20 ± 0.05^{b}	410.32^{b}
Microwave	8.12 ± 0.07^{b}	400.72^{b}
Fluidized bed	5.22 ± 0.05^{c}	320.65^{c}

a–c: Means having a different subscript within a column differ ($p < 0.05$).

Effects of drying process on sensorial properties of pineapple slices are given in Table 4. Drying method is affected sensory properties of pineapple slices significantly ($p<0.05$). Vacuum dried and microwave dried pineapple slices took higher texture and overall impression scores than fluidized bed roasted pineapple slices. However, appearance and flavor values of fluidized bed dried pineapple slices higher than others. This result could be explained by long drying time of vacuum drying technique.

Table 4. Sensory Properties of pineapple slices

Drying method	**Appearance**	**Texture**	**Flavor**	**Overall impression**
Vacuum	3.90 ± 0.12^{c}	4.92 ± 0.14^{a}	4.86 ± 0.18^{a}	4.95 ± 0.02^{a}
Microwave	4.96 ± 0.22^{a}	4.25 ± 0.12^{a}	3.20 ± 0.15^{c}	4.90 ± 0.03^{a}
Fluidized bed	4.22 ± 0.15^{b}	3.50 ± 0.22^{b}	4.10 ± 0.32^{b}	4.10 ± 0.01^{b}

a–c: Means having a different subscript within a column differ ($p < 0.05$).

Conclusion

This study investigated the effect different drying methods on the color, rehydration capacity, shrinking ratio, antioxidant activity, total phenolic content and sensory properties of pineapple slices. The shortest drying time was achieved using the microwave drying method compared to the vacuum drying and fluidized bed drying. Fluidized bed drying caused a decrease in antioxidant activity and total phenolic content of pineapple slices

more than vacuum drying and microwave drying ($p < 0.05$). Rehydration capacity and shrinkage ratio of vacuum dried and microwave dried pineapple slices are higher than fluidized bed dried pineapple slices. On the other hand, vacuum dried and microwave dried pineapple slices took higher texture and overall impression scores than fluidized bed roasted pineapple slices. This study indicated that microwave drying shortens the drying time and can improve the overall quality of the dried pineapple slices.

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Author Information

Sibel Bolek
University of Health Sciences, Department of Food Technology, Üsküdar, İstanbul/Turkey
Contact E-mail: *sibel.bolek@sbu.edu.tr*

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The Use of Molecular Biology Methods in Evaluating Hematologic Diseases

Mehmet OZASLAN
Gaziantep University

Sibel BAYIL OGUZKAN
Gaziantep University

Abstract: Leukemia is a malignant disease originating from lymphopoietic or hematopoietic stem cells of bone marrow. Leukemias are classified as acute or chronic, based on the spread and development characteristics of the tumor. Leukemias exhibit phenotypic and genotypic heterogeneity according to their classification. Therefore, hematology is one of the sciences that most frequently use molecular biology tests. Although the classification and risk assessment are mainly based on cytogenetic analysis, molecular tests play a complementary role. The most commonly used tests include conventional karyotyping, fluorescent in situ hybridization (FISH), polymerase chain reaction (PCR)-based single nucleotide polymorphism analyses (RFLP, ARMS etc.), comparative genomic hybridization (CGH), and sequence analysis methods. Molecular biology tests have become essential for the final diagnosis of malignant diseases, as well as determining the prognosis and even selection of treatment methods. Although cytogenetic and molecular indicators play a key role in determining the risk status of patients, there are also other prognostic indicators in long-term remission. The treatment response can also be evaluated by carrying out morphological, cytogenetic and molecular biology tests (MBT) on bone marrow samples collected at various times throughout the treatment period. In practice, MBTs can be used in the diagnosis and follow-up of benign hematologic disorders (hemoglobinopathies etc.) as well as malignant diseases. MBTs are also frequently used in the diagnosis of congenital or acquired hemolytic anemias, hemophilia, thrombophilia and platelet disorders. Therefore, it is possible to say that the genetic parameters obtained using different MBTs are of utmost importance in the diagnosis, treatment and follow-up period of prevalent malignant or benign hematologic diseases.

Keywords: Hematology, Leukemia, MBT, PCR

Introduction

Hematology is a science that focuses on blood cells, blood-forming organs and the diseases of these organs. Millions of erythrocytes, leukocytes and platelets are produced in the human body each day in order to replace blood cells that are lost within the normal cell cycle (1). Hemostatic mechanisms somehow increase leukocyte production as a response to stress triggers such as bleeding and infection, wherein these cells return to normal levels when stress disappears. Hematopoiesis is the formation of blood cells and maintains the balance in a highly organized manner (2). In hematopoiesis, the fate and differentiation path of a cell is controlled by growth factors or growth factor receptors, specific transcription factors, and the microenvironment (3).

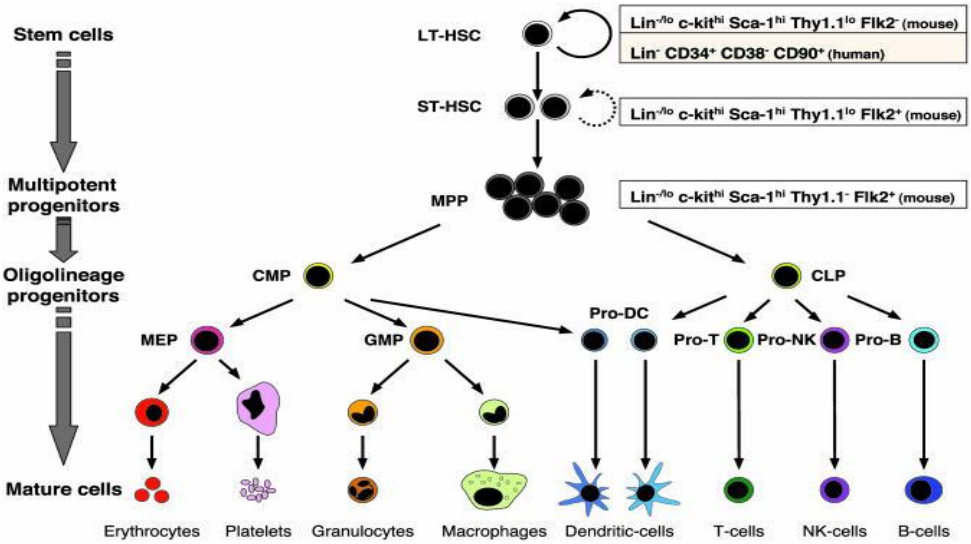

Figure 1. Hematopoiesis (4)

If the hematopoiesis process works as it should, hematopoietic stem cells differentiate into peripheral blood cells (mature lymphocytes, granulocytes, monocytes, erythrocytes and megakaryocytes/platelets etc.) as shown in Figure 1 (5,6). In leukemia however, the cell proliferation rate increases during the stages of bone marrow development cells in one cell line, wherein neoplastic clones proliferate within the bone marrow and start to replace other bone marrow cells. The increase in the number of cell lines is sufficient to replace peripheral blood cells (7). Leukemias constitute a large group of malignant hematologic diseases that are characterized by neoplastic cells covering the bone marrow and other tissues and accumulate in peripheral blood as a result of clonal proliferation, or discontinuation of a specific stage of normal myeloid or lymphoid hematopoiesis. The etiology of leukemia is not entirely known and leads to death if left untreated (8,9).

Leukemias are divided into two groups, i.e. acute and chronic, depending on maturity and survival characteristics (10). Immature hematopoietic or lymphoid progenitor cells are dominant in acute leukemias. There is accumulation of leukocyte progenitors and scarcity of mature cells. Leukemias are divided into two groups, i.e. Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL), according to the structural, cytochemical, immunologic and cytogenetic characteristics of the proliferating cells (11,12). Chronic lymphoid leukemia (CLL) is a type of cancer that develops as a result of the accumulation of small lymphocytes of mature appearance from the morphological aspect. CLL develops as a result of the accumulation of monoclonal cell surface antigens, in other words B lymphocytes, in the blood, bone marrow, lymphoid tissue and spleen (13). Since leukemia is a complex group of diseases that exhibit both phenotypic and genotypic heterogeneity, genetic evaluation is very important in determining the risk level and management of patients, wherein the majority of leukemia patients present with recurring chromosomal abnormalities. Although cytogenetic analyses are essentially used for the classification and risk assessment of leukemias, molecular biology tests play a complementary role (14).

Therefore, this review will evaluate the important molecular biology tests which are currently used and can be used in the future, that are widely accepted in the evaluation of hematologic diseases.

Biological methods used in the diagnosis and follow-up of leukemias

Studies concerning molecular genetics in leukemias started with the identification of recurring chromosomal abnormalities in this disease. Studying the genes with altered/impaired functions as a result of translocation helped to determine the main control pathways of hematopoietic cells and understand how they caused leukemogenesis when impaired. As a result of translocations, either 2 opposite chromosomes may become juxtaposed, thus causing the creation of a new oncogenic fusion gene with the combination of the relevant genes. Alternatively, reciprocal translocation may enhance the function of a gene on one side, causing it to acquire oncogenic characteristics, or result in the gene losing its existing suppressive properties, thereby rendering it unable to prevent the cells from leukemogenesis (15,16).

Cytogenetic studies are primarily necessary in order to investigate the structural or numeric chromosomal abnormalities of leukemic cells. It is accepted that conventional karyotyping is still the first method of choice for this purpose. Fluorescent In Situ Hybridization (FISH) technique, which is a molecular cytogenetic method, and the molecular biology method (MBT) provide results when chromosome analyses fail to suffice (17,18). On the other hand, sub-microscopic changes at DNA level, such as point mutations, can only be detected by molecular methods. Genetic changes shown by these methods play an important role in the diagnosis, classification, determination of subtypes and prognosis of hematologic neoplasia, as well as treatment selection and minimal residual disease (MRD) monitorization. With this purpose, the entire genome is studied to detect mutations using next generation DNA sequencing technologies (19,20). In order to correctly identify these genetic abnormalities that have high clinical significance, it is necessary to first provide the suitable material and then select the suitable method. Therefore, a comparison of the cytogenetic and molecular biology methods used in the diagnosis and treatment of leukemias is provided in Table 1 (21).

Table 1. Comparison of the cytogenetic and molecular biology methods used in leukemias

Properties	**Karyotyping**	**FISH**	**PCR**	**CGH**	**SNP**
Does it ensure the study of the entire genome?	YES	NO	NO	YES	YES
Can it show translocation?	YES	YES	YES	NO	NO
Can it show point mutation?	NO	NO	YES	NO	NO
Can it show deletion?	YES	YES	YES (qPCR)[2]	YES	YES
Can it show amplification?	YES	YES	YES (qPCR)[2]	YES	YES
Can it show rare mutations?	NO	YES	YES	NO	NO

As seen in the Table 1, karyotyping is still the gold standard and other MBTs should be applied as complementary to the Karyocytogenetic Methods (KM), when KMs do not provide results or when the karyotype is normal.

Use of molecular biology methods in benign hematologic disorders

Hematology is a science that investigates not only malignant diseases but also various disorders known to be benign. Erythrocyte disorders can be classified as anemias, thalassemia and sickle cell disease, spherocytosis and G6PD deficiency, acquired hemolytic anemias, erythrocytosis and other congenital hemolytic anemias. Benign leukocyte disorders consist of granulocyte function disorders, granulocytopenia, lymphopenia, lymphocyte function disorders, and leukocytosis. Platelet disorders include acquired platelet function disorders, hereditary platelet disorders, other thrombocytopeniae, and ITP (immune thrombocytopenic purpura). An important group of these benign diseases, i.e. hemoglobinopathies and globin gene disorders, is a large group that encompasses various diseases (sickle cell anemia and thalassemia etc.) (22). Hemoglobinopathies are considered as recessive monogenic (single gene) diseases that are prevalent both globally and in Turkey, with an increasing global burden each year. Nearly 7% of the world's population carries a globin gene mutation. The most common type is beta thalassemia, which is mainly seen in Mediterranean countries, followed by Southeast Asia, India, Africa, Central America and Middle East countries. The incidence of hemoglobinopathies is 2.1% in Turkey, ranging between 0.7% and 13% depending on the region (23). Hemoglobinopathies are systemic diseases. This brings new genetic approaches to the diagnosis and treatment of hemoglobinopathies, although they are monogenic diseases. In order to diagnose and treat hemoglobinopathies completely, it is required to prepare a global map detailing the distribution of the relevant globin gene mutations; reveal the relationship between molecular genetic infrastructure and mutation types, prenatal diagnosis and preimplantation genetic diagnosis, mutation and phenotype; and to be familiar with the pharmacogenetic, immunogenetic, nutrigenetic and gene therapy approaches for globin metabolism and drug use (24,25). Despite the technological advances in detecting mutations, hematologic and molecular biology techniques should be used together in order to reach the correct diagnosis in screenings for hemoglobinopathies.

Therefore, it is necessary to interpret the hematology results and confirm the genotypes using methods such as DNA analysis or mass spectrometry in order to make the correct diagnosis in a majority of carrier screening tests. These methods would be beneficial when hematology or biochemistry results are unclear. In addition, recent studies involving fetal DNA in maternal plasma and state of the art precise technologies, such as digital

PCR and next generation sequencing, provide the routine prenatal diagnosis of globin gene disorders in a manner noninvasive to the fetus (26,27).

Conclusion

New revolutionary discoveries in molecular biology enhance the quality of life and prolong the lifespan of leukemia patients, in addition to providing benefits in various fields. In addition to the advances in molecular biology, close cooperation between the laboratory and clinical findings also play an important role. This review aimed to convey the current molecular biology methods that are frequently used in the diagnosis, screening and follow-up of both malignant and benign important hematologic diseases. It is apparent that technological advances in genetic diagnostics achieved using molecular biology methods, would bring molecular diagnostics to a better position as a fast and inexpensive whole that encompasses the genome sequence of patients.

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Author Information

Mehmet Ozaslan
Department of Biology, University of Gaziantep, Gaziantep, Turkey
Contact E-mail: *ozaslanmd@gantep.edu.tr*

Sibel Bayil Oguzkan
Department of Medical Services and Techniques, Health Services, University of Gaziantep, Gaziantep, Turkey

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Histopathological Study in Patients with Enlarged Thyroid gland

Ilham Abd Allah Ali AL-SALEEM
Al-Mosul University

Wasnaa Abdul KAREEM
Al-Kansaa Teaching Hospital

Abstract: The aim of this research was to scrutinize the histomorphological evaluation of thyroid lesions in linkage to age and sex of the individuals. Materials and methods: This study evaluate the information from 46 thyroidectomy specimens received from october2018 to April 2019 at the Department of cytology, Al-khansaa teaching hospital and laboratory of Mohammed Abdul–Aziz Hayawi. Results: among these 38(82.60%) were from females and 8 (17.39%) were from males with male to female ratio 1:4.5 .The patients age ranged from 1 to 70 years . The large number of lesions are non-neoplastic, found 82.60% (n=38) cases and neoplastic lesions found 17.39% (n=8) cases. Non toxic goiter was the majority lesion approximately 68.42% of all non-neoplastic lesions and about 56.5% of all thyroid lesions. Among other non-neoplastic lesions 5 patients of thyroiditis and three patients of thyroglossal duct cyst were showed. There were 1 malignant tumors and 7 benign tumors in neoplastic lesions. Through the non malignant tumors 3 were follicular adenoma and 1 was Hurthle cell adenoma. Medullary carcinoma was cancer type occur in 1 patients. Conclusion: Our research confirms that non neoplastic type are much widespread in this organ biopsies in contrast to neoplastic type.

Keywords: Thyroid diseases, Hashimoto thyroiditis, Lymphocytic thyroiditis, Focal thyroiditis, Thyroglossal cyst

Introduction

Thyroid gland found in front of the trachea and immediately beneath the larynx on each side (Guyton & Hall, 2006).It secretes calcitonin, thyroxine (T4) and triiodothyronine (T3) hormones (Tsegaye & Ergete, 2003).

Thyroid diseases are serious because most are docile to medical surgical or management. These diseases are accompanied with mass lesions of the thyroid, hyperthyroidism, hypothyroidism (De Benoist, Mclean, Andersson& Rogers,2008).

There are many patterns of thyroid diseases one of it is thyroid neoplasm which classified in two types. The popular non malignant tumor of neoplasm thyroid diseases is adenoma. Thyroid cancer is the other kind of neoplasm thyroid disease, which is the most hesitancy endocrine malignancy (Rahman etal,2013 **)**.

Depending on the statistics data of United States cancer in 2012, malignant thyroid is thought to be the fifth most prevalent recognize malignant in female. Occurrence of malignant this organ is further popular in Saudi Arabia as contrast to the countries in westerner world. It is consider the 2nd widespread cancer followed breast malignant in women (Boone, Fan & Hanna, 2003 **).**

Autoimmune thyroid disease (AITD) is the extreme widespread autoimmune in this organ defect involving of Hashimoto's thyroiditis (gotirous) atrophic thyroiditis, thyroid accompanied ophthalmopathy, postpartum thyroiditis, Graves' disease and silent thyroiditis.These may appear consecutively and sometimes synchronously, in the same patient. These defect portion antibodies against TSH receptor, thyroglobulin (Tg Ab) and thyroid peroxidase (TPO Ab).It is commonly affected middle age men and women. Over to 1% of men and approximately 4% of women are influenced worldwide. It is more popular with forward age; more than 10%

population of age above 75 years is affected. Ultrasonographic appearance, clinical appearance, FNAb findings and autoantibody status are very useful in recognition of AITD(Trbojević & Djurica, 2005 **)**.

One of the popular autoimmune disease is Hashimoto's thyroiditis, which leads to the death.The infiltration of lymphocytic thyroid follicles is the pathophysiological characteristic of this disease. This disease consider as a model not solely for this organ disease, but also for the rest autoimmune disease in the human body (Parvathaneni , Cheriyath , & Fischman ,2012 **).**

Graves' disease is an autoimmune disease recognize by ophthalmopathy, diffuse goiter, hyperthyroidism, and, scarcely dermopathy. Although thyroid-stimulating hormone (TSH) screening has important for the recognition of this disorder, a raise consciousness of heterogeneous and even exemplary presentations is important. In spite of all the obtainable medication effectively normalize thyroid function, each is accompany with dangerous side effects. In guiding the individuals to a remediation resolution, the practitioner should not only be conscious of the prompt risks and advantage of treatment but should also deem near to best maintain the individual's long-term metabolic health (Ginsberg, 2003) **.**

The so prevalent thyroid disease in everywhere of the sphere is non-toxic goiter and is suppose to influence approximately 200 million persons. It is commonly widespread in high land regions but also appears in non-high land places distant from sea (Cotran, Kumar, & Robins, 1994) . There is two style of non toxic goiter an endemic goiter, which happening in a special geographic local places is higher than 10% of the individuals. It is so popular_in everywhere of the sphere and widespread in mountainous regions. A decreased absorption of iodine is lead to this disorder. The other style called sporadic goiter is numerous low prevalent than the endemic type (Tsegaye &Ergete 2003)**.**

The study shed light on the histomorphologic and hesitancy style of thyroidectomy biopsies, study the influence of age and gender as risk factor for shrinking thyroid diseases, to recognize intricacy and relation circumstance of thyroid diseases.

Materials and Methods

Our research is retroactive action in Dept. of Histo-cytopathology of Al-kansaa teaching hospital and the laboratory of Mahmoud Abdl-Aziz Hayawi, Mosul, Iraq. The study period was six month from October 2018 to April 2019. This study were involved 46 biopsies of thyroid. The containment standard were whole biopsies of thyroid of any sex and age while exception standard was deficient and biopsy of thyroid that autolysed. The biopsies were fixed in 10% formalin approximately 3days. Then the fixed samples were displayed to concentration grade of alcohol 70%, , 90% then the absolute alchohol, then submerge in xylen twice then embedded in paraffin, Sectioning in the rotary microtome to 5-micron thickness , placed in water bath (40 - 45 C°) and placed it on slides then Staining method (Haematoxylin and Eosin stain) .

Photography:

Photography was done in college of medical veterinary of AL-Mousl University.
using digital camera with D.C.500 mega pixels.

Results

The Demographical image of patients

Allocation of thyroid disease in study group depending on their age:
(46) biopsies of thyroid were collected in the Dept. of histo-cytopathology of Al-Kansaa teaching hospital and the laboratory of Mahmoud Abdl-Aziz Hayawi, Mosul , Iraq from October 2018 to April 2019.The patients age extend from 1 to 70 years.

The top hesitancy of the individuals were in the fourth decade (n=16; 34.78%), then the third decade (n=11;23.91%).The large number of the thyroid lesions (n=27;58.69%) were showed in the age group 30-49 and 40-49 years. The small number of age group (70-79years) (n=1;2.17%) table(1).

Table 1. Histopathological styles of thyroid biopsies by age group

Type of thyroid diseases	<20	20-29	30-39	40-49	50-59	60-69	70-79	Total	Relative frequency
Nontoxic	3	2	6	11	4	0	0	26	56.5%
toxic	0	1	2	1	0	0	0	4	8.69%
Hashimoto thyroiditis	0	0	1	1	0	0	0	2	4.34%
Lymphocytic thyroiditis	0	0	1	0	0	0	0	1	2.17%
Focal thyroiditis	0	0	0	1	0	0	1	2	4.34%
Thyroglossal cyst	2	0	1	0	0	0	0	3	6.52%
Adenoma	1	2	0	2	2	0	0	7	15.2%
Carcenoma	0	0	0	0	1	0	0	1	2.17%

Allocation of thyroid diseases of study group depending on their gender:

As in present study, there were 38 (82.60%) females and 8 (17.39%) males and the ratio of female to male 4.75:1 .See table (2).

Table 2. Histopathological pattern of thyroid tissue by sex group

Types of thyroid diseases	**Male**	**Female**	**Female: Male**
Nontoxic	2	24	12:1
toxic	1	3	3:1
Hashimoto thyroiditis	0	2	2:0
Lymphocytic thyroiditis	0	1	1:0
Focal thyroiditis	0	2	2:0
Thyroglossal cyst	3	0	0:3
Adenoma	2	5	2.5:2
Carcinoma	0	1	1:0

Allocation of thyroid diseases depending on the non-neoplastic and neoplastic organ diseases.

In the current research, the hesitancy of non-neoplastic and neoplastic the organ biopsies is about17.3% and about 82.6% respectively. The non-neoplastic biopsies are prevalent in 3rd to 5th decades of life table(3).

Table 3. Allocation of non-neoplastic and neoplastic thyroid disease.

Type of thyroid disease	**Hesitancy**	**Relative hesitancy**
Non-neoplastic	**38**	**82**.6%
Neoplastic	**8**	**17**.3%

Microscopic appearance of thyroid gland in individuals with thyroid diseases

In the patients of non toxic colloid goiter sections display thyroid follicles filled with colloid material lined by flat epithelial cells contained in a well defined cyst wall

In the case of non toxic colloid goiter (Multinodular hyperplasia) the sections of thyroid glands appear proliferation of variable sized follicular cells also accompanied with areas of hemorrhage, calcification and fibrosis figure (1).

In the patients with toxic adenoma the sections appear thyroid tissue with follicles contain pale colloid and some fine vaculated with scalloping, the size of follicles is combination of pattern degenerative changes seen between follicles the tissue surrounded by total thin capsule, figure (2), (3).

In the case of hashimmoto thyroiditits the section appear benign acini with Hurthel cell metaplastic alterations and thyroid paranchyme infiltrated by plasma cells lymphocyte some of them germinal centre figure(4),(5).

In the biopsy specimen of non toxic colloid goiter with focal lymphocytic thyroiditis the section appear thyroid follicles of different sizes lined by flat epithelial cells filled with colloid material associated with scattered areas of fibrosis and focal lymphocytic aggregates figure(6).

In the case of lymphocytic thyroiditis section show different size follicles filled with colloid with nodule formation, fibrosis and inflteration by heavy growth of lymphocyte figure (7).

In the case of thyroglossal cyst the sections of thyroid show pseudo stratified ciliated columnar epithelium with underlying mucosa containing vascular channels of different sizes mixed with few inflammatory cells and remnant of thyroid follicles embedded within skeletal muscles, figure (8).

In the cases of follicular adenoma of thyroid the sections show closely packed small follicles lined by cuboidal epithelium with pale staining nuclei and round inconspicuous nuclei enclosed in acompletely enveloped thin fibrous capsule associated with scattered degenerative changes figure(9).

In the case of medullary carcinoma, sections show solid proliferation of mixtures of round, polygonal and spindle cells arranged in nests and cords associated with low mitotic figures separated by a highly vascular and hyalinized collagen stroma.

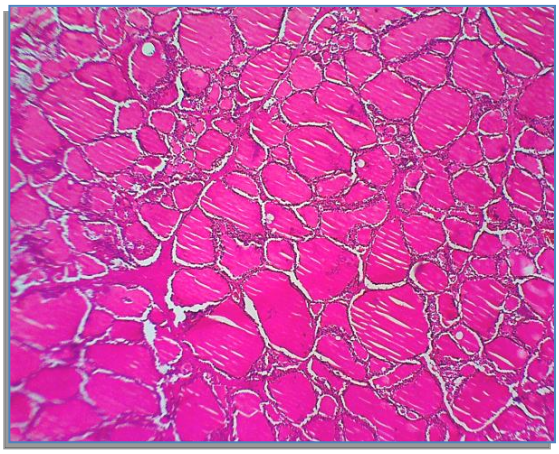

Figure 1. Photomicrographs of thyroid gland in individuals with non toxic goiter. (H&E4X)

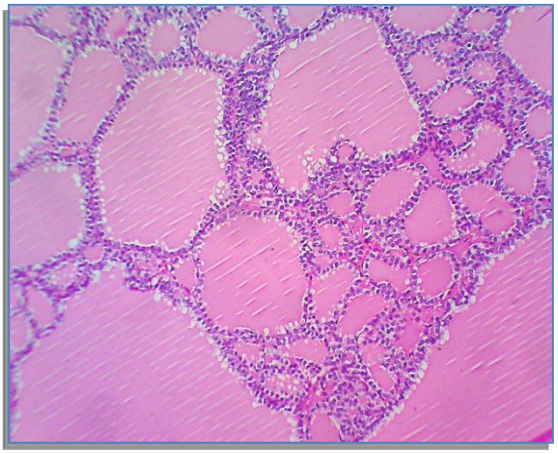

Figure 2. Photomicrographs appearance of thyroid gland in individuals with toxic goiter. (H&E4X)

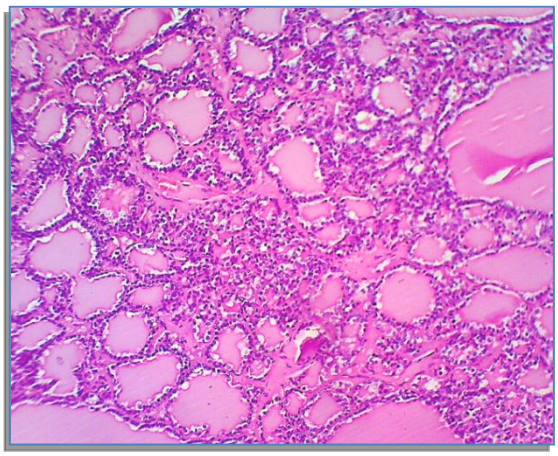

Figure 3. Photomicrographs of thyroid gland in individuals with toxic goiter. (H&E4X)

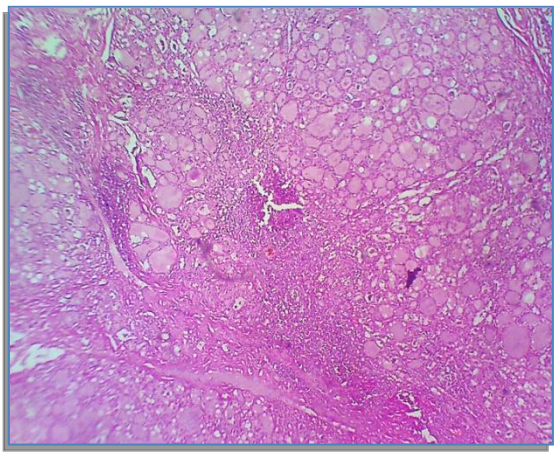

Figure 4. Photomicrographs of thyroid gland in individuals with Hashimoto thyroiditis (H&E4X)

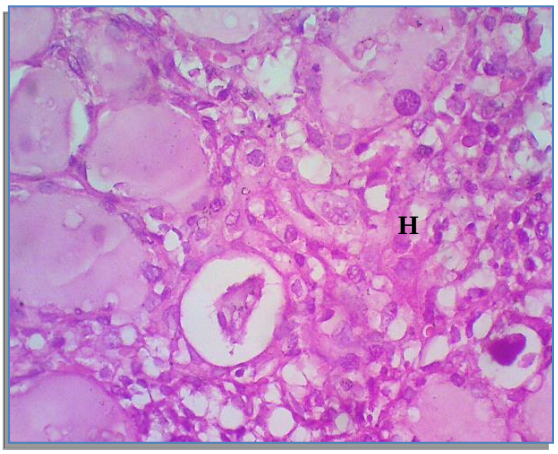

Figure 5. Photomicrographs of thyroid gland in individuals with Hashmoto thyroiditis show the hurthel cell **H**. (H&E 40X)

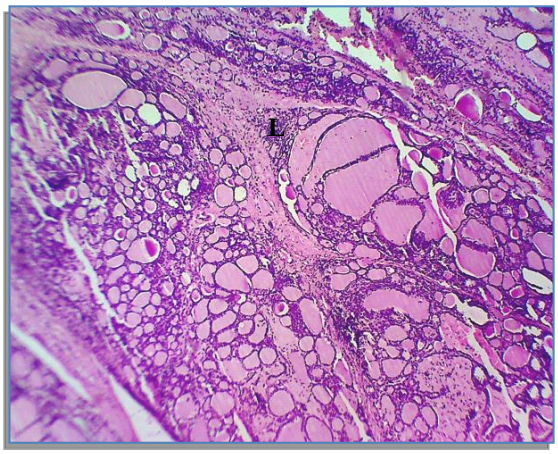

Figure 6. Photomicrographs of thyroid gland in individuals with lymphocytic thyroiditis show the lymphocyte infilteration **L(**H&E4X).

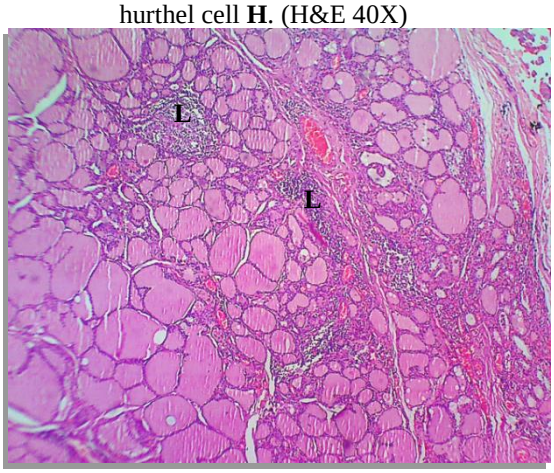

Figure 7. Photomicrographs of thyroid gland in individuals with focal lymphocytic thyroiditis show the focal lymphocyte aggregation. **L**(H&E10X).

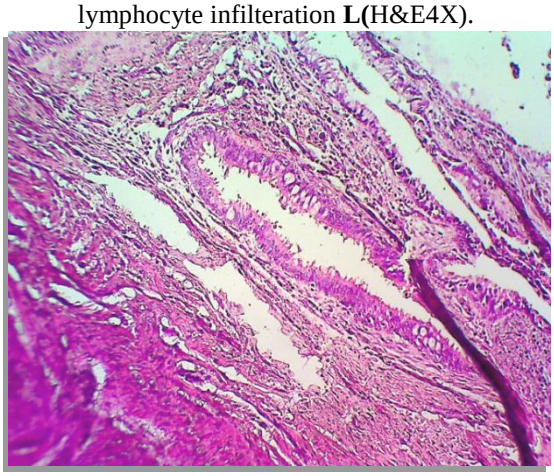

Figure 8. Photomicrographs of thyroid gland in individuals with thyroglossal cyst. (H&E10X).

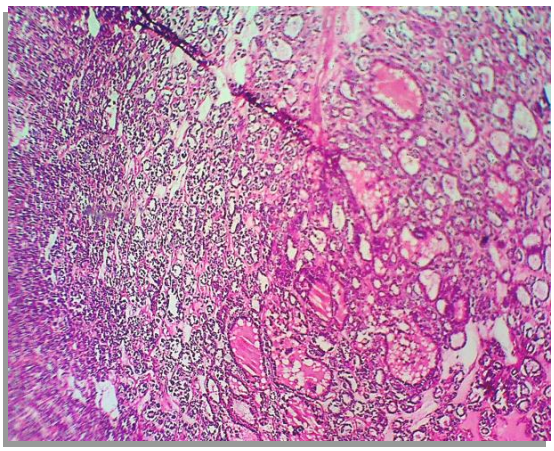

Figure 9. Photomicrographs of thyroid gland in individuals with follicular adenoma. (H&E4X).

Discussion

In our research the age of the individuals extend from 1 to 70 years. The top hesitancy of the individuals were in the fourth decade (n=16; 34.78%), followed by third decade (n=11; 23.91%).The larger number of the thyroid defects (n=27; 58.69%) were noticed in the age group 30-49 and 40-49 years. The small number of age group (70-79 years) (n=1;2.17%).

This study was in agreement with other previous research that showed the age of the individuals extend from 1to 74 years with an average age 35.57 ± 12.65 years. The larger number of the thyroid diseases (n=86; 79.63%) were noticed from 21 to 50 years. The age of young series (<20 years) and the age of old seies over 60 years formed 2.78% and 10.19%Consecutively (Rahman et al ,2013).

Regarding to the gender, the present study demonstrated that there were38 (82.60%) females and 8 (17.39%) males the ratio of female to male about 4.75 :1.

This study agreed with another study that noticed that thyroid diseases is more common in females approximately (80.5%) than males (19.5%) (Tsegaye & Ergete, 2003).The other studies confirmed that (Gitau, ,1975;Kungu, 1974; Mekones, 1996;Wolde-Gebriel , Demeke , West , &van der Haar ,1993). Also another study certained that thyroid diseases have been occurred in female more than male (Bokhari & Sadiq 2008)**.**

Depending on non neoplastic and neoplastic biopsies, the current paper found that the hesitancy of non-neoplastic and neoplastic biopsy cases is about82.6%and 17.3%. The non-neoplastic cases are popular in 3rd to 5th decades in females in contrast to males. **.**

The present paper confirm other studies that notice that non neoplastic lesions were about 82.85% and neoplastic lesions about 17.14% cases (Abdul Ghafoor, Sajjad, Akram,& Khan,2015)**.**

In the type of non toxic goiter**,** this research demonstrated that the non toxic goiter is prevalent in the age between**(**30-39**)** and **(** 40-49)years in percentage 56.5%.

This results is lower than the previous study found that Nodular colloid goiter consider prevalent defect like it was found in approximately 600(76.9%) of biopsies in their research.

The grade of the increase in the size of thyroid is commensurate to the period and level of the thyroid hrmon reduction. In the majority of specimen the elevated thyroid mass fulfill euthyroid condition(Tsegaye & Ergete ,2003).Colloid goiter was the most common lesion, compute as 56.5% of all thyroid lesions and 68.4% of all non-neoplastic lesions. This high hesitancy was also confirm by others. (Adeniji, Anjorin, &Ogunsulire , (1998;Bokhari & Sadiq, 2008; Imran, Majid, Khan,2005;Tsegaye & Ergete ,2003) **.**

In the type of toxic goiter, we found this type of disease in 4cases (8.69%).Other studies performed by Hussain et al found 0.91% of cases (Hussien, Anwar, Nadian ,& Zulfiq, 2005 **).**

In the present study, out of the 46 cases studied, inflammatory lesions group contained 5 patients (10.86 %). Of these, the most dominant inflammatory lesion was Hashimoto and focal thyroiditis (40%) and lymphocytic thyroiditis (20%).

Our research was in agreement with another research that confirmed that out of the 100 specimens studied, inflammatory lesions' group contained 8 cases (8.0 %) (Monika, Meena, &Sayajirao, 2018) **.**

Hashimoto thyroiditis included of 4.34% of total cases, all being females. Our results was inagreement in another research that demonstrate the percentage is approximately 2.6% of whole biopsies and all being females (Abdul Ghafoor, Sajjad, Akram & Khan Z.A, 2015).

The low hesitancy and the female sex tendency over males in thyroiditis were also confirm by other studies. (Kumar, Abbas, Fausto ,& Aster, 2010;Tsegaye & Ergete, 2003)**.** Where is our results HT was diagnosed in three to fourth decades.

This result was inagreement with another reseach that confirmed that (HT) which is an autoimmune inflammatory defect diagnosed by prevalent fibrosis, lymphocyte cell infiltration, oxyphilic changes and thyroid

cell atrophy that influence of about 5% of the individuals but is appeared in the 4th to 6th decennium and is about 15 once more prevalent in female (Cipolla et al,2005).

The results of this study notice the thyroglossal cysts in three (6.52%)cases. The current research was in resumble with other research which demonstrated that the disease appear in four patients (3.7%) of thyroglossal duct cyst were found (Rahman et al,2013).

In the adenoma type ,the current study of was demonstrated in 15.2% of individuals. Our results was in agreement with other study that found the benign tumor example, adenoma were noticed in about 100 (12.8%) biopsies (Tsegaye & Ergete, 2003),and also found that the hesitancy of follicular adenoma is more than the hesitancy of cancers **(**AL Bouq, Fazili , &Gaffar,2006; Tsegaye & Ergete, 2003 **).**

The present study also found that females are so prevalent influenced than the males this results was match with other previous study(Abdulkareem, 2010; Salama, Abdullah, Al-Qahtani, &Al-Maghrabil 2009).
In cancer style, there is a relatively decrease widespread of thyroid malignancy (TM) was notice about 2% (1/46) of only medulary carcinoma.

A study found that medullary thyroid carcinoma (MTC) involves 5 – 10% of whole this organ cancers. The average age at coming is 50 years (Salama, Abdullah, Al-Qahtani, &Al-Maghrabil 2009) **.** This is in harmony to our study in which MTC involves 2.17% with average age of 54.5 years, which may refers that our case were intermittent styles**.**

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Author Information

Ilham Abd Allah Ali Al-Saleem
Department of Biology, College of Science,
Al-Mosul University,
Mosul, Iraq.
Contact E-mail: *ilhsbio41@uomosul.edu.iq*

Wasnaa Abdul Kareem
Department of Histo-cyto Pathology,
Al-Kansaa Teaching Hospital,
Mosul, Iraq.

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Diagnosis of Achromobacter xylosoxidans and Klebseilla oxytoca as Etiological Agents of Peptic Ulcers

Shadan A. AL-WENDAWI
Baghdad University

Abstract: Helicobacter pylori is the pathogen only known that inhabits the gastric mucosa of almost half of the world's population, and the bacterium is associated with higher incidence of peptic ulcer worldwide. The present study was aimed to seek for diagnosis of H. pylori as etiological agents of peptic ulcer in Iraqi patients on second line therapy and suffering from severe ulcer reinfection after a period of time. Sixty-five endoscopic gastric biopsy specimens were obtained from patients of both genders and in age around 45-60 years. For primary isolation, 26 (40%) out of all corpus and antrum screened biopsies were positive on supplemented Columbia agar. Culture isolates showed heteroresistance pattern to antibiotics used in triple therapy regimen for eradication of H. pylori infections, in that high percentage of resistance to tetracycline and metronidazole (100%) was recorded, while most of isolates were sensitive and in various degrees (27%, 12.5%, and 25%) to amoxicillin, clarithromycin, and levofloxacin respectively, on the other hand one isolate exhibited absolute resistance to all of the tested antibiotics. The molecular detection of 16S rRNA (109bp) and ureA (411bp) genes specific for H. pylori were not detected by PCR amplification. Two isolates which showed significant similarities to H. pylori throughout the morphological and cultural examination, were selected and subjected to molecular analysis via 16S DNA sequencing. GenBank BLAST analysis was showed that the isolates were non-H. pylori isolates, rather, one was identified as Achromobacter xylosoxidans and the other Klebsiella oxytoca, with 98% and 97% identical gene sequences respectively. .

Keywords: Helicobacter pylori, Colombia agar medium, Standard triple therapy, Proteobacteria, Klebsiella oxytoca, Achromobacter xylosoxidans

Introduction

Helicobacter pylori is spiral shaped, Gram-negative microaerophilic microorganism, naturally found in more than 50% of the world's human population who are asymptomatic and healthy (1), with fewer than 15% of the carriers developing disease like gastroduodenal diseases such as duodenal and gastric ulcers. Furthermore, the bacterium could be the trigger of various malignant diseases of the stomach (2). Quick evolution of multidrug resistant isolates and no vaccine until today lead to global spread of H. pylori and cause worrying health disorders worldwide especially in developing countries (3). In respect of isolation and diagnosis of H. there are many noninvasive diagnosis methods, such as the antibody-based tests like stool antigen test, serological diagnosis and urea breath test (4). And several invasive (direct) tests, which is routinely most followed once. These tasting depends on mucosal endoscopic biopsies , such as rapid urea test, histopathological sections examination and laboratory cultivation. Cultivation based on using of specific and highly selective bacteriological culture media (5). The endoscopic gastric biopsies cultivation must achieved in 2-8% O2, 10% CO2, and incubation for more than 3 days. Cultural strains are identified, through means such as, Gram staining, catalase, oxidase and urease as one of classical, effective and sensitive diagnosis procedure for H. pylori (6). Although the culture method is a "gold standard" for diagnosing many infectious diseases, it is not easy in the case of an H. pylori infections diagnosis. The invasive cultural methods sensitivity of the H. pylori isolation shows a marked variation and now decreased, owing to the small population of bacteria that colonizes the stomach, and two or three biopsy specimens from different locations are requested for accurate diagnosis (7). Moreover, the positive cultural results are not always guaranteed and easily vanished under normal atmospheric conditions (8). Therefore, a number of molecular detection procedures have been developed that have been extensively used, the PCR methods that involves several housekeeping genes like; 16S rRNA, rpoD, ureA,

ureB, and ureC to detect H. pylori in biopsy specimens, and culture isolates (9, 10). The optimal treatment for H. pylori infections has not been yet found. Clinical treatment for eradicate of H. pylori infection, routinely is combination therapy, triple or quadruple antibiotic therapy and remains the worldwide choice for eradicate H. pylori infections in patients with both gastric and duodenal ulcers (11). Triple therapy involves combination of two different antibiotics (clarithromycin or metronidazole, amoxicillin, tetracycline or rifabutin), plus proton pump inhibitors (PPI) for 7 to 14 days (12). The eradication cure rates of this dependent regimen are relatively low as 50 – 70 % (13), due to the high rates of antibiotic resistance (14). Know it was noticing the PPI based treatment for more than two weeks changes the stomach pH to more alkaline conditions, and this may cause inflammation of the stomach lining and induced glandular atrophy (15). The documented studies referred, the elimination of H. pylori encouraged some other opportunistic microorganisms to thrive, enabling more severe gastric infections and gastritis to spread to the upper compartment of the stomach, which could ultimately promote the development gastric cancer (16). Currently studies have been shed light on the impacted association of H. pylori with human stomach microbiota. It was reported abundance of non-helicobacter bacteria, particularly from Proteobacteria (to which H. pylori belongs) in gastric microbial profiles of H. pylori positive patients (17). In vivo Murine model studies supported the role of non-H. pylori, particularly proteobacterial members in the development of gastritis from peptic ulcers to gastric neoplasia (18). Such studies in positive H. pylori patient's demonstration has been showed gastric environment neutralization, and this closely correlated the alterations in the gastric microbiota and significantly increased colonization of proteobacteria (19).

The aim of this study was to seek for the prevalence of H. pylori isolates as etiological agent for peptic ulcer in patient suffering from prolonged recurrent peptic ulcer, by using the cultural and molecular detection procedures.

Materials and Methods

Specimen Collection

Sixty-five endoscopic gastric biopsy specimens were taken by specialist from corpus and antrum region of patient's stomach in age of 45 – 60, who were previously diagnosed with peptic ulcers. The study enrolled patients were attended the Gastroenterology Department/ Baghdad teaching hospital, Baghdad/ Iraq. All patients were on second line ulcer antibiotic therapy and not recovered after months of massive medication. The obtained biopsies were placed in separate tubes containing sterile phosphate buffer saline (PBS) pH 7, and transported to the laboratory within 1-2 hrs.

Cultivation of Bacteria

The biopsy specimens were gently ground and homogenized, a loop full of the homogenate mixtures were streaked on supplemented Columbia agar (Micromedia/Hungary), agar base enriched with 7% (v/v) horse blood and supplemented with Dent's supplement, which is a combination of three different antibiotics; amphotericin B 5 µg.ml.-1, trimethoprim 5 µg.ml-1, and vancomycin 10 µg.ml-1 (20). The plates were incubated in 95% humidified atmosphere with 10% CO2 at 37°C, for 6-7days. Pronounced colonies were sub-cultured on Columbia agar and brain heart infusion supplemented with 5% (v/v) sheep blood. The colonies were identified on the bases of colony morphology, Gram staining, catalase, oxidase and urease testing results.

Antibiotic susceptibility

The antibiotic susceptibility profile against routinely used antibiotics in triple therapy regimen for eradication of H. pylori, including amoxicillin 2µg (AMO,), tetracycline 30 µg (TET), metronidazole 5 µg (MET), clarithromycin 15µg (CLT) and levofloxacin 5µg (LEV), was performed using the disk diffusion method (Kirby–Bauer) on Muller Hinton (MH) agar medium (Himedia/ India). In brief; fresh overnight bacterial cultures in brain heart infusion broth were adjusted to McFarland tubes No. 0.5, (108 CFU/ml), and aliquots 100 µl of each culture was individually swabbed on the surface of MH agar, antibiotic discs were gently placed on the agar surface under strictly aseptic conditions, plates were incubated at 37°C for 48 hrs. The inhibition zone diameters around antibiotic disks were measured and interpreted as recommended by the Clinical Laboratories Standard Institute (21). Some of the isolates were not able to grow on the Muller Hinton agar medium, therefore horse blood was added to the culture medium to support growth.

PCR identification of Bacterial isolates

The DNA of the bacterial isolates that thought to be H. pylori, was extracted by using genomic DNA purification kit (Intron Biotechnology, Korea), and according to manufactures instructions. All isolates were subjected to PCR analysis to identification the housekeeping genes. The primer pair Hp1F/HP2R was used to target the 16S rRNA gene specific for H. pylori and HPU1F/HPU2R was used to target the ureA gene (Integrated DNA technologies / Canada). The universal primer 27F/1492R was used to detect the bacterial universal 16S rRNA gene of the bacterial isolates (Macrogen/Korea) as shown in table (1). The reaction programs of PCR are listed in table.2. The program was held at 4 °C, and then the PCR products were visualized by using of gel electrophoresis..

Table 1. Sequence of primers used for identification the gastritis associated bacteria

Gene	Oligonucleotide	Sequence	Reference
16S rRNA specific for *H. pylori*	Hp1F/HP 2R	5′ CTGGAGAGACTAAGCCCTCC 3′ 5′ ATTACTGACGCTGATTGTGC 3′	(22)
ureA gene	HPU 1F/HPU 2R	5′ GCCAATGGTAAATTAGTT 3′ 5′ CTCCTTAATTGTTTTTAC 3′	(23)
universal *16S rRNA* gene	27F/1492 R	5′ AGAGTTTGATCCTGGCTCAG 3′ 5′ CGGTTACCTTGTTACGACTT 3′	(24)

Table 2. Program used for amplify the 16 rRNA, ureA genes, and universal 16 rRNA

Primer	Initial denaturation	Denaturation	Annealing	Extension	Final extension
		40 cycles			
Hp1F/HP2R	95 °C (10 min)	95 °C (30 sec)	55-60°C (1 min)	72°C (1 min)	72°C (5 min)
HPU1F/HPU2R	95 °C (10 min)	95 °C (10 min)	45 °C (1 min)	72°C (1 min)	72°C (5 min)
20F/1530R	95 °C (3 min)	95 °C (45 sec)	62°C (45 sec)	72°C (1 min)	72°C (10 min)

Sequencing and Sequence Alignment

The sequence of universal 16S rRNA gene of isolates 3 and 9 was carried out by sending the PCR products of amplified 16S rRNA gene to Macrogen Company/ Korea to preform Sanger sequencing by using AB13730XL, automated DNA sequences. The result analyzed by BLAST website on NCBI.

Results

Cultivation and preliminary identification

Out of sixty-five analyzed corpus and antrum biopsies, 26 (40%) were pronounced visible colonies on supplemented Columbia agar. The gastritis associated bacterial (GAB), harvested colonies were tiny, pinpoint, transparent, convex, and circular (Fig. 1). The GAB isolates were number coded (1-26). The respective colonies were further taken for Gram staining and microscopic examination, those that stained Gram negative were selected. The shape of the bacteria appeared as long twirled, with varying lengths and seemed to be segmented, with curved rod-wing shapes, it seemed like these long bacterial cells were broken down into pieces forming wing and rod like shapes, spiral shapes were also noticed, and coccoid morphology was dominant in old cultures that were kept at 4°C for a week or more.

All GAB isolates were catalase and oxidase positive, while, only eight isolates have been positive for urease, all the urease negative isolates were eliminated from study due to mismatch with the control.

Antibiotic susceptibility

The GAB isolates antibiotic susceptibility was analyzed against five antibiotics belonged to four class, and they included to standard triple therapy. The susceptibility patterns are summarized in table, 3. Out of 8 GAB isolates susceptibility were evaluated, one isolate (isolate 3) exhibited multiple resistant pattern giving the overall Multidrug resistant (MDR) rate of 12.5%. While, the rest of the isolates were susceptible in various degrees (27%, 12.5%, and 25%) to AMO, CLA, and LEV respectively. On the other hand, all GAB isolates were recorded absolute resistance rate (100%) to both of MTZ and TET.

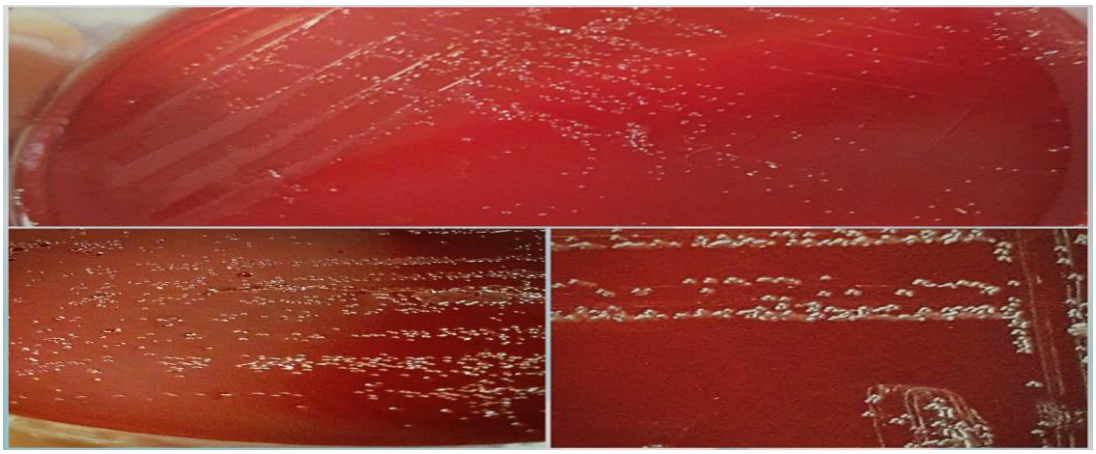

Figure 1. The gastritis associated bacteria isolates colonies on supplemented Colombia agar

Table 3. Antibiotics susceptibility test for gastric associated bacterial isolates

Antibiotics	Break point	Inhibition (mm)	Resistance (%)
Amoxicillin (25 µg)	**≥21 < 16**	12-28	3 (37.5%)
Metronidazole (5 µg)	**≥ND < 21**	9-17	8 (100%)
Tetracycline (30 µg)	**< 17**	8- 15	8 (100%)
Clarithromycin (15µg)	**≥ND < 21**	19-33	1 (12.5%)
Levofloxacin (5 µg)	**≥19 < 17**	15-29	. 2 (25%)

PCR identification of Bacterial isolates

Eight GAB isolates that gave the same characteristics of H. pylori under the microscope, colony morphology, and biochemical testing results were included to molecular detection through PCR amplification of H. pylori 16S rRNA (109bp) and ureA (411bp) genes. After multiple opportunities of running PCR, trying different techniques, changing protocols, and DNA extraction methods, no genes were detected, and no isolate gave positive results (Fig. 2). The isolates 3 and 9 which showed significant morphological similarities to H. pylori and were recovered from the biopsies of severe illness cases, were subjected to identified by amplification of the bacterial universal 16S rRNA gene (Fig.3).

The alignment for the sequence of isolate 3 was revealed high matching with the universal strain (CD-253) sequence which is recorded on NCBI as Achromobactyer xylosoxidans species (accession number: JQ724537.1) at 97% query cover of 98% identify and 0% gaps.

As for the variations seen for the alignment of sequence isolate 9, was revealed high matching with the universal strain (Pb41) sequence which is recorded on NCBI as Klebseilla oxytoca species (accession number: KU761531.1) at 100% query cover of 97% identify and 1% gaps.

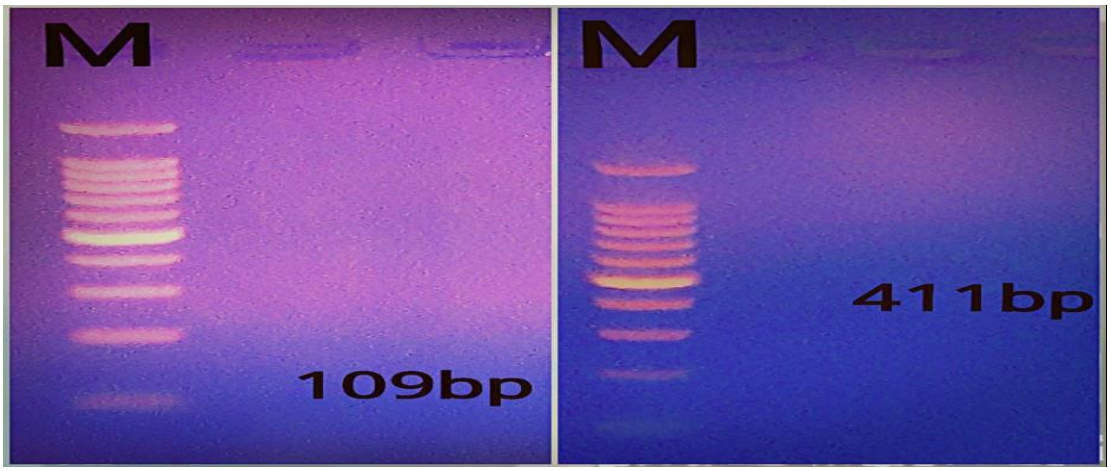

Figure 2. Electrophoresis of PCR product for amplified 16S rRNA and ureA genes of the "thought to be H. pylori" isolates, M-100bp Ladder, A. negative results no 16S rRNA gene detected B. also negative results no ureA gene detected. Electrophoresis was performed on 1% agarose, 70volt/cm for 90min.

Figure 3: Electrophoresis of PCR product ~ 1500 bp of the universal 16S rRNA gene, M-100bp Ladder, Lane (1) isolate 3, Lane (2) isolate 9. Electrophoresis was performed on 1% agarose, 70 volt/cm for 90min.

Discussion

Helicobacter pylori infection represents a key factor in the etiology of various gastrointestinal diseases ranging from asymptomatic gastritis to stomach carcinoma (2). For best management of H. pylori related diseases, the accurate diagnosis is required particularly for infection treatment course. There are various diagnosis methods used for diagnosis of H. pylori in different subjects, precisive detection of this bacterium in different obtained clinical specimens corresponding to the successful therapeutic practice's strategies. Traditionally noninvasive and invasive diagnosis techniques are followed, and each one has certain advantage and disadvantages (5). The invasive diagnosis are endoscopic biopsy dependent methods, and noninvasive are followed to avoid endoscopy (4). Nevertheless, cultural invasive diagnosis based on using highly selective media with several supplements to support bacterium growth and thrive, due to the fastidious and slow growth rate of this bacterium, and its special incubation conditions, such as 10% CO2 and high humidity (6). Also, great care is required during collection and transportation of suspected biopsy specimens to insure successful isolation. Several selective agar media have been used for the isolation of H. pylori from fresh biopsies. Columbia agar medium is one of the most frequently recommended base media to propagate H. pylori culture in routine diagnosis (22,23). Columbia agar supplemented with 7% fresh horse blood used as a base medium with the addition of Dent's supplement, which used to inhibit contaminated flora without loss of H. pylori recovery (20). Multiple biopsies samples rather than a single antral biopsy needed in order to increase the sensitivity and specificity of culture in the diagnosis of the H. pylori (24). Antibiotic resistance is the main reason for failure to eradicate H. pylori infection, the antibiotic resistance of H. pylori has significantly changed over time (25). The obtained GAB isolates were included to the

susceptibility testing based on traditional first line triple therapy regimens used for eradication of H. pylori (26). The obtained pattern of resistance to MET and TET was unexpected even though the resistance to antibiotic drugs may change over time in addition to the resistance depending on the geographical area (27), but the wide shift toward absolute resistance is abnormal, should not be considered for therapy due to the recorded high resistance rate (100%) to both antibiotic. The globally recorded resistance rate to MET does not exceed 80% (28). A study reported discrepancy between in vitro MET resistance and treatment outcome, this may be explained by the changes that occur in the oxygen pressure in the gastric environment, as MET resistant H. pylori isolates become MET-susceptible under low oxygen conditions in vitro (29). As well as, the absolute resistant rate recorded to TET was not in accordance with the publications as the resistance rate of H. pylori to TET is very low (07- 1%) or even absent in most cases (30). The CLA and AMO has resistance amongst the thought to be H. pylori isolates can be considered relatively low. Studied in large scale over the last years have been recommended CLA the drug of choice, although the resistance to CLA seems to be increasing in many geographical areas and the age of the patient affected the resistance rate to CLA (31). The resistance rates to LEV is within normal range 25%, The rates of resistance to metronidazole and clarithromycin are increasing worldwide (32). Based on many publications, standard triple therapies may not be recommended anymore for treatment, due to the high level of resistance to the two key antibiotics of standard triple therapies, CLA and MET, and the different patterns of resistance in different populations (33, 34, 35). Therefore, not considering the resistant isolates to these antibiotics would be incorrect. The discrepancy between the result of this study and the other published studies is due to the consequence of non-isolating H. pylori, rather isolating other bacterial species from the biopsy specimens which was confirmed by molecular diagnosis tools as explained later.

To entitle a sample as H. pylori positive current evidences are indicating the requirement of at least one other positive test in addition to the culture positive result (23) The approach of using PCR genetic invasive identification technique has been proposed, as reliable and predictive confirming diagnosis. However, having positive result from a specific PCR approach can easily replace those time-consuming and expensive tests (36). H. pylori is a microorganism with marked genetic diversity rely using H. pylori housekeeping genes for accurate identification and to evaluate the causes of gastroduodenal diseases (37). Un expected genotyping results was obtained from PCR analysis, not being able to detect the genes (16S rRNA and ureA) of H. pylori, means the ulcer biopsies isolated bacteria may be not H. pylori, or may be the obtained biopsy specimens were not taken from the exact stomach ulcerative area, due to the sensitivity of diagnosis techniques depends on the observer's experience and extent of biopsy sampling (38). Another important issue should be taken in concern, the target gene primer sets not always designed properly, and mis-diagnosis may attribute in some extent to variability in different DNA extraction protocols (39), In this context, a universal approach needs to be recommended to produce reliable results, at least in clinical settings. As one of global housekeeping genes, 16S rRNA was used, (40). However, recently the sequencing of the 16S rRNA gene present in all bacterial species has been a useful tool for identification, it is time consuming and gives accurate results having the16S rRNA gene a molecular marker that is general for all the members of this domain. It's more reliable due to the misidentification of microorganisms when using cultural techniques (41). The 16S r RNA sequencing, GenBank BLAST analysis was showed that the isolates were non-H. pylori isolates, rather, one was identified as Achromobacter xylosoxidans and the other Klebsiella oxytoca, with 98% and 97% identical gene sequences respectively. These two bacteria were isolated from gastric walls of infected patients at regions with ulcers, the patients recruited for the study, were complaining of how they have cured their ulcer infection and then got severe re-infected again after a period of time in addition to the massive amounts of medication they were consuming. May be H. pylori died throughout the first line medication which was a combination of antibiotics and PPI, usually taken for 14 days to eradicate H. pylori. The antibiotics cannot function in the acidic environment of the stomach, therefore, PPI is included within therapy regimen to reduce the hydrochloric acid secretion and the gastric juice acidity (42). This reduces antibiotics washout, thereby increasing luminal antibiotic concentration which causes the lining mucosal layers of the GIT fully damaged. After the course of medication, a completely damaged mucosal membrane was left with no viable H. pylori bacteria in addition to the alkaline stomach conditions there remained months after PPI was stopped (43), and this improves the GIT microbiome and environmental pathogens chance to infect the damaged mucosa. Recently many studies have established links between non-H. pylori microbiota and peptic ulceration, and this is part of studies focuses on the relationship between GIT microbiome in some systemic diseases (44). The ones infected with H. pylori are known to be have a higher content of bacteria that belong to the phylum Proteobacteria in their stomach (17), A. xylosoxidans, K. oxytoca, and H. pylori belong to the Proteobacteria. Studies show that people with dysbiosis have inflamed regions throughout the GIT due to antibiotic therapy, have increased epithelial oxygenation which disturbing the anaerobes and leads to an increase in the facultative anaerobiosis (45). Most of the studies that showing A. xylosoxidans isolated from clinical specimens are from patients with cystic fibrosis (46). One possible way for this bacterium can gain stomach colonization, could be from the sputum swallowed by a patient with an A. xylosoxidans lung infection and cause infection in the stomach (47). Achromobacter is highly motile bacterium

that prefers humid environments and can survive in extreme environments such as, the heavy doses of antibiotics (48), and infects those persons with non-protecting lining epithelia and weak gastric mucosa, allowing it to cause infection and eventually gastritis. 15-year time period studies were supported the role of non-H. pylori microorganisms in the development of chronic gastric ulcer to malignant tumors, and highlighted the effects of H. pylori eradication therapy on gastric neoplasia (49). A computerized search algorithm was construct to identify the presence of bacterial DNA within interrogated known cancer genomes, they identified that the type of cancer that harbored the second highest number of bacterial DNA sequences was gastric adenocarcinoma, and found the most common type of bacterial DNA was not H. pylori but instead was Pseudomonas, and due to their high similarity A. xylosoxidans is always misdiagnosed with Pseudomonas (50). The fact that one of the isolates turned out to be A. xylosoxidans not H. pylori, the break points for resistance was not available by the CSLI, instead it was considered one of the organisms that should not be monitored "Unusual organisms which are rarely involved in serious infections" (WHO, 2011). Studies used the breakpoints recommended by CLSI for non- Enterobacteriaceae (51). A study was performed to demonstrated the MDR profile of human derived strains of A. xylosoxidans, intrinsically it was resistance to list of study included therapies, (benzylpenicillin, cefoxitin, cefamandole, cefuroxime, glycopeptides, fusidic acid, macrolides, lincosamides, streptogramins, rifampicin, daptomycin, and linezolid) (52). This proves the extremophilic behavior of bacterium that stays alive while all the other bacteria in the stomach or intestine, which will give them an opportunity to cause infection. Our isolate showed clear resistance to all the antibiotics tested with no inhibition zone.

The seconded isolated bacterium (isolate 9) turn to K. oxytoca, numerous studies have shown that H. pylori eradication second line therapy may cause antibiotic associated hemorrhagic colitis (AAHC), where patients begin with a peptic ulcer and after the repeat courses of medication end up with more severe AAHC. Clinical studies were approved predominance of Klebsiella. oxytoca in biopsy specimens taken from such patient from inflamed colon tissue (53). Recently K. oxytoca has been isolated at a significantly high rate from endoscopic biopsy was taken from severe inflamed gastric mucosal necrotic areas for elderly man, was diagnosed H. pylori positive. Contrast-enhanced computerized tomography scan of abdomen demonstrated diffuse gastric wall thickening and an intramural abscess in the gastric antral wall (54). Genome sequencing of K. oxytoca clinical isolate was revealed a combination of virulence, antibiotic resistance and metabolic genes that assist the species survive and transmit within the human clinical isolate in host and overcome challenges in the harsh habitat such as antibiotics (55). This was realized throughout this study during the antibiotic susceptibility test where the isolates that ended up to be K. oxytoca showed variable results, a few times showing light growth surrounding the CLA and AMO discs, and other times sensitive with inhibition zones which may consider them resistant to these antibiotics resulting in their resistance to the full triple therapy and other times showing a clear inhibition zone. Both of these unusual bacterial isolates had a mucoid appearance, in which this characteristic helps in protection from the tough conditions of the stomach and large intestine enabling its capacity to produce biofilm and surviving throughout the GIT. The ability of a bacterium to produce biofilm is dangerous owing to its difficulty in eradication and having the mucus lining of the GIT suitable for such act (56).

Conclusions

A combination of at least two diagnostic tests with high sensitivity and specificity for diagnosis of peptic ulcer etiological agents.

Ulcerative gastritis is not always related to infection with H. pylori, may be other bacteria such as GIT microbiota or external opportunistic bacteria are the causative agents.

Abuses of H. pylori eradication therapy may consequently change stomach microbiome and lead to more severe ulceration cases

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Author Information

Shadan A. Al Wendawi
College of science/ Biology Dept. / Baghdad university.
Baghdad / Iraq
Contact E-mail:

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Evaluation the levels of Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), Calcium and Phosphorus in Iraqi non-Hodgkin lymphoma patients treated and untreated

Zahraa Mohammed Ali HAMODAT
University of Mosul

Nuha A. AL- TALİB
University of Mosul

Abstract: Non-Hodgkin lymphoma (NHL) is a disorder caused by malignant change of lymphocytes that affect the lymph nodes or extra-node areas. Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) play an important role in non-Hodgkin lymphoma.Our current study aims at estimating levels of lactate dehydrogenase, alkaline phosphatase, calcium and phosphorus in Iraqi non-Hodgkin lymphoma patients treated and untreated.Patients and Methods: Our study included 120 people, including 80 people with non-Hodgkin (34 females, 46 males, ages 25-70 years) were visiting the Oncology Hospital in Mosul, Iraq. And 40 uninfected people were considered as a control group, identical in age (19 females, 21 males). In addition, the patients were divided into two groups, one under treatment and the other newly diagnosed (untreated). Serum lactate dehydrogenase, alkaline phosphatase, calcium and phosphorus were measured.The results of our study showed that the average age of patients was 56.1±11.1 years, and the average age of healthy persons is 52.2±13.8 years. The results indicated that non-Hodgkin lymphoma patients have a significant increase (P<0.0001) in levels of lactate dehydrogenase (529±164.5 U/L, NHL patients versus 299.1±34.5U/L controls) and alkaline phosphatase (298±115.7 U/L NHL patients versus 98.8±39.4U/L controls) compared to healthy people. Also, the results indicated that untreated NHL patients have a significant increase (P< 0.0001) in levels of LDH (697.6±292.3 untreated NHL patients versus 317.6±92.3 treated NHL patients) and ALP (336.3±122.4 untreated NHL patients vs. 112.3±42.3 treated NHL patients) compared to treated NHL patients.While no significant difference was shown in the levels of LDH, ALP, calcium and phosphorus between the groups of treated and healthy people.It is possible to use lactate dehydrogenase and alkaline phosphatase as an indicator to diagnose and to know the severity of the disease as well as to monitor the effect of treatment for patients.

Key words: Non-Hodgkin lymphoma, Lactate dehydrogenase, Alkaline phosphatase

Introduction

Lymphoma is a type of slag disease, characterized by the proliferation of malignant cells of the lymphatic system. Lymphoma is classified into two distinct types: Non-Hodgkin's Lymphoma (NHL) and Hodgkin's Lymphoma (HL), (Galleze et al., 2017; Yildirim, Gundogdu, & Erdem, 2009; Zinzani, 2005).

non-Hodgkin lymphoma is a disorder caused by malignant change of lymphocytes that affect the lymph nodes or extra-node areas (Purnamasidhi, Suega, & Bakta, 2019). The incidence of the contract outside the lymph nodes is 24-48%. The incidence has increased since the previous decade, and areas outside the lymph nodes may occur in any organ, and the most commonly occurring areas are the digestive system, skin and bones. (Lu et al., 2013; Yadav et al., 2016). Non-Hodgkin lymphoma affects males more than females (50-75%), and it affects between 60 and 70 years of age. (Yadav et al., 2016).

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme that stimulates the reverse reaction of pyruvate to lactate, which is the last step in the glycolytic (Galleze et al., 2017; Long et al., 2019; Purnamasidhi, Suega, &

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Bakta, 2019) (Galleze et al., 2017). It is a widespread enzyme in various tissues such as liver cells and muscles such as the heart muscle. (Galleze et al., 2017; Purnamasidhi et al., 2019; Yadav et al., 2016). Also, the activity of lactate dehydrogenase increased in heart patients and types of cancer such as pancreatic and colon cancer(Galleze et al., 2017; Lu et al., 2013). Galleze et al., 2017 which has showed a significant rise of lactate dehydrogenase in non-Hodgkin's lymphoma patients, but the activity of lactate dehydrogenase in non-Hodgkin Lymphoma (NHL) is higher than Hodgkin lymphoma (HL) (Galleze et al., 2017).

Alkaline phosphatase (ALP) belongs to the enzyme group that activates the decomposition of mono-aster phosphate. It is capable of interacting with various foundation materials, ALP is found in all tissues of the body, especially in the membrane, found at a high level in the bone renal tubes, the liver Intestinal epithelium and placenta(Tahannejad, Dayer, & Samie, 2012; Taher, Hummadi, AL-Bashir, & AL-Araji, 2016). Calcium is a major structural component of the skeleton(Kalita & Choudhury, 2017; Kumar & Bhaskar, 2012).

Our current study aims at estimating levels of lactate dehydrogenase, alkaline phosphatase, calcium and phosphorus in Iraqi non-Hodgkin lymphoma patients treated and untreated.

Materials and Methods:

Our study was conducted in Mosul city through the period from the 1st of May of 2018 to the 1st march in 2019. Our current study included 120 people, including 80 people with non-Hodgkin (34 females, 46 males, ages 25-70 years) were visiting the Oncology Hospital in Mosul, Iraq. In addition, the patients were divided into two groups, one under treatment and the other newly diagnosed (untreated). All patients have written information included: medical history, physical examination related to history of non-Hodgkin lymphoma, age, gender, and family history of non-Hodgkin lymphoma. Cases were excluded from the study such as: Hodgkin lymphoma (HL) and other disease.

Also, forty healthy people aged- 25-70 years (19 females, 21 males) were considered as a control group. 5 milliliters (5ml) were pulled from the vein and placed in a clean tube and centrifugeated at 3000xg and then separated the fine part of the serum samples were kept under freezing -20 until the tests were performed.Serum lactate dehydrogenase, alkaline phosphatase, calcium and phosphorus were measured.

Estimation the level of lactate dehydrogenase (LDH) activity: The activity of lactate dehydrogenase (LDH) was measured using a factory kit by Bay Labo (French). The principle of measurement includes that LDH stimulate the internal transformation of the pyruvate converted to lactate and the presence of NADH as coenzyme, Optical absorption measured at 340 nm(Burtis, Ashwood, & Bruns, 2012).

Estimation the level of Alkaline phosphates (ALP)activity: Alkaline phosphate activity (ALP) was measured according kind and king method(Kind & King, 1954) by using a factory kit by Biomerex (French). The principle of measurement includes the measurement of phenol released from the interaction of potassium ferricyanide and 4- aminoantipyrine.

Estimation the level of calcium: calcium was measured in the spectrophotometric method and using a factory kit by Bay Labo (French). The principle of measurement involves the interaction of calcium with O-Cresolphthalein (CPC) at alkaline medium. Formation a pink color complex, which measured at 570 nm (Burtis et al., 2012).

Estimation the level of phosphorus: Phosphorus was measured in the spectrophotometric method and using a factory kit by Bay Labo (French). The principle of measurement involves the interaction of interaction of ammonium molybdate with phosphomolybdic complex, in acid medium, releasing phosphorus ion. The absorbance was measured at λ= 340 nm (Burtis et al., 2012).

Statistical Analysis: The statistical analysis was carried out using statistical programe (SPSS). The results expressed a mean± SD (standard deviation). Consider significant when P values are less than 0.05 (Beddo & Kreuter, 2004).

Results and Discussion

The results of our study showed that the average age of non-Hodgkin lymphoma patients (NHL) was 56.1±11.1 years, and the average age of healthy persons (controls) was 52.2±13.8 years as shown in Table 1.

Table 1. Clinical Distribution of Patients (A)

	Controls	Patients (NHL)
Total number (Sex (F/M))	(19/21)	80 (34/46)
Treated patients (Sex (F/M))	-	40 (18/22)
Untreated patients(Sex (F/M))	-	40 (16/24)
F: Females; M: males; NHL: non-Hodgkin lymphoma.		

The results as shown in Table 2. indicated that non-Hodgkin lymphoma patients have a significant increase (P<0.0001) in levels of lactate dehydrogenase (529±164.5 U/L, NHL patients versus 299.1±34.5U/L controls) and alkaline phosphatase (298±115.7 U/L NHL patients versus 98.8±39.4U/L controls) compared to healthy people.

The results showed the high lactate dehydrogenase for patients and this is consistent with (Gandhi, Pophali, & Witzig, 2019)Galleze et al., 2017; Lu et al., 2013; Tahannejad, Dayer, & Samie, 2012(Gandhi et al., 2019))This is probably due to the fact that in non-hodgkin's lymphoma patients gets in an increase in the rate of sugar decomposition (GLYCOLUYSIS) and thus increases the effectiveness of lactate dehydrogenase, thus it is possible to consider LDH as a tumor index for lymphatic non-Hodgkin's patients. (Galleze et al., 2017; Shamoon & Polus, 2010).

Also, the results indicated that untreated NHL patients have a significant increase (P< 0.0001) in levels of LDH (697.6±292.3 untreated NHL patients versus 317.6±92.3 treated NHL patients) and ALP (336.3±122.4 untreated NHL patients vs. 112.3±42.3 treated NHL patients) compared to treated NHL patients as shown in Table 3. Increase in LDH activity possibly due to the use of too much glucose in metastatic tissues and increased glucose conversion to pyrophosphate(Tahannejad et al., 2012; Taher, Hummadi, AL-Bashir, & AL-Araji, 2016).

While no significant difference was shown in the levels of LDH, ALP, calcium and phosphorus between the groups of treated and healthy people as shown in Table 4.Also, increased significant (P<0.0001) level of alkaline phosphatase in NHL patients compared with the controls were agreement with (Tahannejad, Dayer, & Samie, 2012). The high level of activity of ALP may probably be attributed to the high use of too much energy by cancer cells. The results of the study also showed that there is non-significant difference in the level of calcium and phosphorus between patients and healthy people.

Table 2. Comparing the variables studied between the patients of the non-Hodgkin lymphoma and the healthy subjects (B)

Variables	Controls	Patients (NHL)	p-value
Age (no.)	52.2±13.8 (40)	56.1±11.1 (80)	0.122
LDH (U/L)	299.1±34.5	***529±164.5	0.0001
ALP (U/L)	98.8±39.4	***298±115.7	0.0001
Calcium (mg/ml)	8.1±1.3	8.3±1.6	0.09
Phosphorus (mg/ml)	3.6±0.6	3.7±0.73	0.13
NHL: non-Hodgkin lymphoma; LDH: lactate dehydrogenase; ALP: alkaline phosphatase; mg: milligram; ml: milliliter; U: unit no.: number. ***significant at (p≤ 0.0001) when p value be at (0.0001).			

Moreover, the results indicated that untreated NHL patients have a significant increase (P< 0.0001) in levels of lactate dehydrogenase (697.6±292.3 versus 317.6±92.3) and alkaline phosphatase (336.3±122.4 vs. 112.3±42.3) compared to treated patients, as shown in Table 3.

Moreover, the results also showed a significant increase of lactate dehydrogenase in untreated non-Hodgkin lymphoma compared to therapists, while there is no significant difference to the activity of lactate dehydrogenase among patients treated with healthy people and this is consistent with (Galleze et al., 2017; Purnamasidhi et al., 2019) which means LDH can be used as an indicator of the response to chemotherapy and the improvement of patients' health. The increased activity of lactate dehydrogenase reflects the prevalence of

the tumor, as the increased activity of LDH (Purnamasidhi et al., 2019). Also in the advanced stages of the disease the shattered tissues increase and as a result gets more liberalization of ALP and LDH enzymes that explain the results we have obtained in untreated patients. While no significant difference was shown in the levels of calcium and phosphorus as shown in Table 3

Table 3. Comparing the variables studied between treated and untreated non-Hodgkin lymphoma (D)

Variables	Patients (NHL) (treated)	Patients (NHL) (untreated)	p-value
Age (no.)	52.4±10.5 (40)	6.1±10.4 (40)	0.913
LDH (U/L)	317.6±92.3	***697.6±292.3	0.0001
ALP (U/L)	112.3±42.3	***336.3±122.4	0.0001
Calcium (mg/ml)	8.2±1.1	8.3±1.35	0.075
Phosphorus (mg/ml)	3.65±0.53	3.75±0.73	0.85
NHL: non-Hodgkin lymphoma; LDH: lactate dehydrogenase; ALP: alkaline phosphatase; mg: milligram; ml: milliliter; U: unit no.: number. ***significant at (p≤ 0.0001) when p value be at (0.0001).			

While no significant difference was shown in the levels of lactate dehydrogenase, alkaline phosphatase, calcium and phosphorus between the groups of treated and healthy patients as shown in Table 3.

Table 4. Comparing the variables studied between treated non-Hodgkin lymphoma and healthy (C)

Variables	Controls	Patients (treated)	p-value
Age (no.)	52.2±13.8 (40)	54.4±10.5 (40)	0.913
LDH (U/L)	299.1±34.5	317.6±92.3	0.289
ALP (U/L)	98.8±39.4	112.3±42.3	0.155
Calcium (mg/ml)	8.1±1.3	8.2±1.1	0.075
Phosphorus (mg/ml)	3.6±0.6	3.65±0.53	0.85
NHL: non-Hodgkin lymphoma; LDH: lactate dehydrogenase; ALP: alkaline phosphatase; mg: milligram; ml: milliliter; U: unit no.: number. ***significant at (p≤ 0.0001) when p value be at (0.0001).			

The results showed that untreated patients get a significant increase (P<0.0001) for ALP and LDH enzymes compared to treated patients, while the results indicated that there was non-significant increase in ALP and LDH for treated patients compared to healthy patients.

The activity of lactate enzyme increases when lymphatic proliferation disorder occurs, in non-Hodgkin's lymphoma patients the level of activity of lactate dehydrogenase increases, so it can be an important predictive indicator and can therefore be used as an indicator to respond to treatment. (Yadav et al., 2016).

Conclusion

The results of our study showed significant increase in the activity of ALP LDH for NHL patients compared to healthy people. Moreover, a significant increase in the activity of ALP LDH for untreated NHL patients compared to treated NHL patients and a non-significant difference of levels of LDH, ALP, calcium and phosphorus between treated NHL patients when compared with healthy patients. It is possible to use lactate dehydrogenase and alkaline phosphatase as an indicator to diagnose and to know the severity of the disease as well as to monitor the effect of treatment for patients.

Acknowledgements or Notes

Please provide acknowledgements or notes in a separate section at the end of the article before the references.

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Author Information

Zahraa Mohammed Ali Hamodat
Assistant professor, PhD, biochemistry, University of Mosul, College of Science, chemistry, 07704764647, Mosul / Iraq
Contact E-mail:*zahorahamodat@gmail.com*

Nuha A. Al- Talib
Lecture, MSC, biochemistry, University of Mosul, College of Science, chemistry,
Mosul / Iraq