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Provide a context or background for the study (i.e.,the nature of the problem and its significance). State the specific purpose or research objective of, or hypothesis tested by, the study or observation; the research objective is often more sharply focused when stated as a question. Both the main and secondary objectives should be made clear, and any pre-specified subgroup analyses should be described. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

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Conclusion should elucidate how the results communicate to the theory presented as the basis of the study and provide a concise explanation of the allegation of the findings.

ACKNOWLEDGEMENT

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PAKISTAN BIOMEDICAL JOURNAL

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Volume 6, Issue 6 (June 2023)

Cancer Research in Pakistan: Progress, Challenges, and Future Directions

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Cancer is a growing public health concern in Pakistan, with rising incidence rates and significant impacts on individuals and communities. In recent years, cancer research in Pakistan has made notable strides in understanding the epidemiology, risk factors, and treatment outcomes of various cancer types. However, numerous challenges persist, hindering progress in cancer control and prevention. Exploring the progress made, acknowledging the challenges faced, and outlining future directions is crucial for advancing cancer research and improving cancer care in Pakistan.

In terms of progress, cancer research in Pakistan has provided valuable insights into the burden of different cancer types across various regions. Studies have shed light on the prevalence of common cancers such as breast, lung, and gastrointestinal cancers, enabling targeted interventions and screening programs. Furthermore, collaborative efforts between research institutions and healthcare providers have resulted in improved diagnosis and treatment modalities, with advancements in radiotherapy, chemotherapy, and surgical techniques.

Despite these advancements, several challenges persist. Limited access to quality cancer care, particularly in rural areas, remains a significant barrier. Insufficient infrastructure, shortage of skilled healthcare professionals, and financial constraints impede timely diagnosis, treatment, and follow-up care. Additionally, cultural beliefs and social stigmas surrounding cancer often lead to delayed diagnosis and inadequate support for patients and their families. Overcoming these challenges requires comprehensive strategies, including strengthening healthcare infrastructure, training healthcare professionals, and raising awareness about cancer prevention and early detection.

Looking ahead, the future of cancer research in Pakistan lies in a multidimensional approach. Enhancing collaboration among researchers, healthcare providers, and policymakers is crucial for effective data collection, research translation, and evidence-based policymaking. Investment in research infrastructure, including molecular and genetic profiling facilities, can facilitate targeted therapies and personalized treatment options. Additionally, fostering international collaborations and partnerships can provide opportunities for knowledge exchange, access to innovative technologies, and clinical trials.

Cancer research in Pakistan has made significant progress in understanding the burden of cancer and improving treatment outcomes. However, challenges such as limited access to quality care and cultural barriers must be addressed to ensure equitable and comprehensive cancer services across the country. By investing in research infrastructure, fostering collaborations, and promoting awareness, Pakistan can enhance cancer research capabilities and make substantial strides in cancer prevention, early detection, and treatment. A concerted effort by researchers, policymakers, healthcare professionals, and communities is necessary to combat the growing burden of cancer and improve the lives of individuals affected by the disease.

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Volume 6, Issue 6 (June 2023)

Review Article

Fenugreek: A Health promoting Food

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ABSTRACT

Lifestyle decisions, food habits, stress, environmental variables, and synthetic substances are all contributing to the rapid advancement of human diseases. It has been demonstrated that the herb fenugreek is effective in treating conditions like cancer, high cholesterol, diabetes, and inflammation. Fenugreek extracts and powders have been successfully used in the culinary and pharmaceutical industries because of its therapeutic properties. This review aim was to emphasize the significant nutritional advantages and therapeutic uses of fenugreek as a strong treatment for a range of diseases.

INTRODUCTION

Trigonella foenum-graecum L., also known as fenugreek, is a significant spice crop utilized in human diets. It can be utilized for nutritional, nutraceutical, medical, and therapeutic purposes because it is abundant in phytochemicals, alkaloids, carbohydrates, steroidal saponins, amino acids, and minerals [1]. It has been employed as an extruded product, an emulsifier and stabilizer in culinary items, and as a flavor enhancer in traditional cuisine. The physiological and nutraceutical benefits of fenugreek, which support its prospective use in creating a variety of pharmaceutical and functional food items[2].

Fenugreek's nutritional value

Fenugreek is a plentiful source of bioactive compounds, including fiber, proteins, carbs, and lipids as depicted in table 1 [3]. Minerals including potassium, magnesium, calcium, zinc, manganese, copper, and iron are also present. Additionally, it contains sulfur-containing amino acids as well as amino acids including aspartic acid, glutamic acid, leucine, tyrosine, and phenylalanine [4]. According to studies, the proteins in fenugreek are of higher quality than those found in other plants.

Table 1: Nutritional composition of fenugreek

Fenugreek	Composition
Iron	3.9mg
Copper	0.2mg
Manganese	0.1mg
Magnesium	22mg
Vitamin B6	0.3mg

Alkaloids, flavonoids, and polyphenols such quercetin, luteolin, vitexin, 7, 4-dimethoxy flavanones, aglycones, kaempferol, quercetin, tricin, and naringenin are also present in substantial amounts in fenugreek as shown in figure 1 [5]. The majority of the flavonoids in fenugreek, according to a phytochemical analysis, occur as

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glycosides, which are complex due to their C- and O-glycosidic bonding with carbohydrates [6].

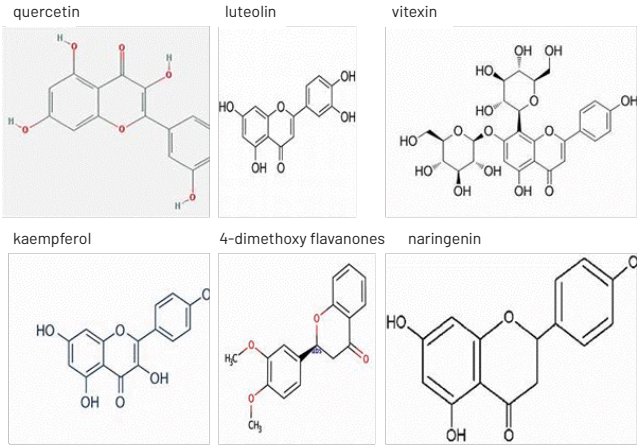

Figure 1: Bioactive compounds of Fenugreek

Therapeutic Effect of fenugreek

Function in managing rheumatoid arthritis

Chronic inflammation is a feature of the joint condition rheumatoid arthritis. In comparison to indomethacin, fenugreek mucilage at 75 mg/kg demonstrated the strongest impact against oedema in rats, according to a study [7]. The study also discovered that the anti-arthritic capabilities of fenugreek mucilage led to reducing the activity of inflammatory enzymes to reduce oedema [8].

Anticancer perspectives

Plant-based active ingredients are utilized to prevent cancer, which is one of the leading causes of mortality worldwide. According to studies, fenugreek seeds can prevent mammary hyperplasia in rats and decrease the proliferation of HL60 cells [9]. Fenugreek (*Trigonella foenum-graecum*) extract from the whole plant has also been discovered to have cytotoxic effects against many human cancer cell lines as shown in figure 2. Despite having no effect on primary or immortalized prostate cells, fenugreek extract has been discovered to have anti-cancer effects, including the ability to slow the growth of cancer cell lines from breast and pancreatic malignancies [10,11]. Fenugreek seed extract demonstrated a 70% reduction in tumor cell proliferation. Fenugreek seed extract decreasing tumor incidence and lipid peroxidation [12].

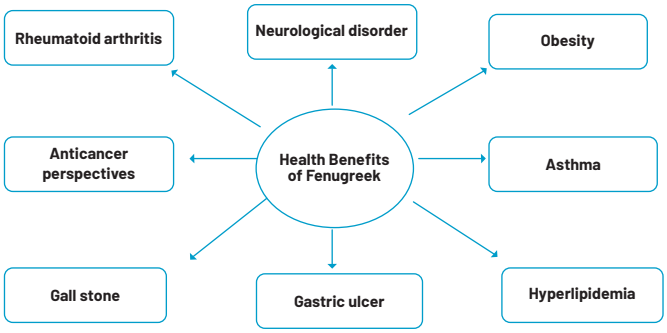

Figure 2: Health benefits of Fenugreek

Fenugreek against gall-stone and gastric ulcer

In a rat model, the antiulcer properties of *Trigonella foenum* seed [13]. They discovered that the aqueous and gel components of fenugreek seeds have an impact on ant secretory activity and mucosal glycoproteins. Additionally, they discovered that the combination of fenugreek and onion had the strongest anti-lithogenic effects, reducing cholesterol gallstones by 76%, 27%, and 75%, respectively, when onion was supplemented with fenugreek [14]. Additionally, it increases total bile acid and biliary phospholipid levels and helps prevent and treat cholesterol gallstones. These disorders respond favorably to active components like flavonoids found in fenugreek seeds, gel, and aqueous extract [15].

Fenugreek against neurological disorders

For the treatment of neurological illnesses like depression, Alzheimer's, and Parkinson's, fenugreek has been studied as a potent medicinal plant [16]. Studies have demonstrated that fenugreek saponins, 5% fenugreek seed powder, and ethanolic extract of fenugreek were effective in lowering the prevalence of Parkinson disease, attenuating depression, and enhancing neurotransmission [17]. Through the use of animal models, the mechanisms behind the antidepressant effects of fenugreek flavonoids. These investigations add to the growing body of research showing that fenugreek components have profound neuroprotective effects [18].

Effect of Trigonella against obesity

Numerous studies have demonstrated that hydroxyl isoleucine reduces insulin resistance brought on by obesity. It reduced the activity of a catalyst that changes tumor necrotic factor from mTNF to sTNF [19]. Additionally, fenugreek's fiber content reduces appetite, which is enhanced in obese experimental units. Fenugreek supplements taken orally have been demonstrated to significantly accelerate weight loss over a short period of time. Fenugreek-derived furanostolic saponins (FenfuroTM) have been shown to improve glucose tolerance, improve insulin sensitivity, and decrease insulin-activated protein kinase B's phosphorylation [20].

Fenugreek in asthma treatment

The 10% heights of FEV1 and FEV1/FVC were increased by fenugreek seed extract, and the serum IL-4 levels were lowered. Flavonoids inhibited the production of Charcot-Leyden and eosinophil cationic proteins, who also have antioxidant capabilities. Asthma patients' results were improved by fenugreek and honey syrups, with the latter being superior to the former when combined. To comprehend its efficacy, more study is required [21].

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CONCLUSIONS

Fenugreek has been found to have nutritional and pharmacological uses, such as antioxidants, anti-inflammatory agents, and clinical applications. Further research is needed to isolate bioactive components, synthesize recombinant pharmaceutical proteins, and conduct clinical studies.

Authors Contribution

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Effect of Atenolol on Hepatic Dysfunction by Evaluating Level of AST

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ABSTRACT

Hypertension is one of the most important risk factors for morbidity and mortality around the world. Many drugs used to treat hypertension have several side effects. Atenolol used to control hypertension, is an anti-beta-adrenergic agent. It has found to significantly affect the hepatic functions. **Objective:** To study the effects of atenolol on hepatic dysfunction by measuring AST level in hypertensive patients, taking atenolol alone or in combination with other anti-hypertensive drugs. **Methods:** The variation in AST level was measured upon use of atenolol alone or in combination with other anti-hypertensive drugs. Out of total 80 patients, 43 were treated with atenolol alone, 37 with atenolol in combination with other anti-hypertensive drugs and 20 were healthy controls. Micro lab tests were used for measurement of AST level. **Results:** Significant results were found as atenolol cause increase in AST level. Other anti-hypertensive drugs did not affect the AST levels much and the increase in AST level was more significant in patients taking atenolol alone. **Conclusions:** Atenolol may have efficacy in controlling hypertension, but it causes disturbance in AST levels therefore any other drug in combination with atenolol is recommended to avoid AST variation and eventually hepatic dysfunctions. Further this study may be employed on large scale for strengthening the outcomes of this study.

INTRODUCTION

Hypertension is a chronic medical condition in which the blood pressure in the blood vessels is too high i.e., 140/90 mmHg or higher [1]. Major causes of hypertension include the complex interplay of pathological conditions including genetic predisposition as well as different environmental factors [2, 3]. Various systems including autonomic nervous system, the renin-angiotensin-aldosterone system, and nitric oxide and endothelin, which are secreted by the endothelium, are the major regulators of blood pressure and control cardiovascular homeostasis [4-6]. Any variation or disturbance in these systems can lead to hypertension. So, these systems are targeted for developing anti-hypertensive drugs. Different anti-hypertensive drugs have been developed to treat high blood pressure, including: alpha blockers, ACE inhibitors, angiotensin receptor blockers, beta blockers, calcium channel blockers, central alpha agonists, diuretics, renin inhibitors and vasodilators [7, 8]. Among these drugs one of the widely use drugs is beta blockers including Atenolol which is a selective β1 receptor antagonist. Atenolol was developed in 1976 as a replacement for propranolol in the treatment of hypertension. Atenolol has the beta-adrenergic receptor blocking activity by reducing the blood pressure at resting and after exercising, inhibition of the tachycardia and reduction of orthostatic tachycardia. Atenolol also decreases the cardiac output without affecting the total peripheral resistance [9]. When atenolol is used to treat hypertension, it has found to be responsible

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for some side effects including hepatic dysfunction. It causes damage to the liver cell and produce a variety of symptoms but the main problem originates from glucose insufficient supply to the brain leading to functions impairment. Liver function tests (LFTs) are performed in clinical biochemistry which indicate the condition of patient's liver [10, 11]. Abnormal liver function tests (LFTs) have been reported in people with high blood pressure [12]. AST (Aspartate transaminase) is a sensitive indicators of liver damage or injury from different forms of diseases. The elevated level of AST indicates the damaged liver however there could also be some others factors responsible for this increase in AST level e.g., muscle damage also causes an increase in AST level in blood its normal range for male lies within <35U/L and for females <31 U/L [13]. This study was based on finding the effect of atenolol on hepatic functions by measuring the AST level in hypertension patients after they are treated with atenolol alone or in combination with other anti-hypertensive drugs.

METHODS

In this study, total of 80 subjects were selected with hypertension taking atenolol alone or in combination and 20 subjects with normal blood pressure were taken as control. Patients were selected from Punjab Institute of Cardiology, Lahore for the evaluation of hypertension status. Those with hypertension taking atenolol alone or in combination were selected and their AST levels were checked. All positive hypertensive patients were included and those on multidrug therapy and with concomitant disorder were excluded in this study. Those with normal blood pressure were taken as control for comparison. After collection of blood from the patients, blood was centrifuged, and serum were separated. The AST level was determined by measuring the change in absorbance with time due to the conversion of NADH to NAD^+ which was measured by using a by using a photometer 5010plus at 340/400nm rate technique. Addition of pyridoxal-5-phosphate (P-5-P), recommended by IFCC, stabilizes the activity of transaminases and avoids falsely low values in sample containing insufficient endogenous P-5-P, from patients with hypertension.

RESULTS

The effect of atenolol on liver was studied by measuring the level of AST in blood of hypertensive patients. Figure 1 indicates the significant change (increase) in AST level in patients treated with atenolol alone (mean value = 49.9) as compared to patients treated with atenolol in combination with other anti-hypertensive drugs (mean value = 42.2) and while the mean value in control patients was 32.

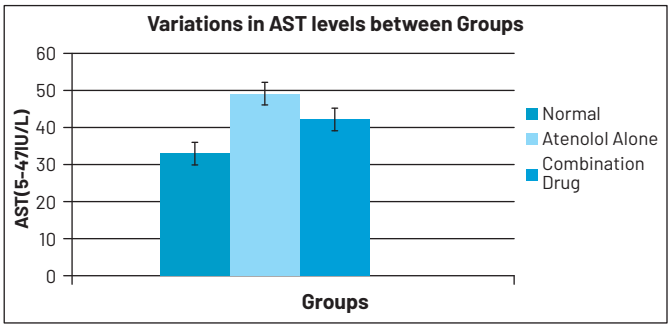

Figure 1: Graphical representation of variation in AST level between patients divided in three groups

In control group of 20 people of random age and gender with normal blood pressure have AST levels in normal range for males lies within <35U/L and for females <31 U/L (Table 1).

Table 1: Evaluation of AST in control population

Sr. No.	Gender	Age	AST
1	Male	35	12
2	Male	45	23
3	Male	37	30
4	Male	48	32
5	Male	51	39
6	Male	36	25
7	Male	42	36
8	Male	41	29
9	Male	55	49
10	Male	34	23
11	Female	35	28
12	Female	44	40
13	Female	45	36
14	Female	46	34
15	Female	44	25
16	Female	55	36
17	Female	35	37
18	Female	33	24
19	Female	57	43
20	Female	42	41

The group of patient's takings atenolol alone having random age and gender have significant disturbance in AST levels as compared to control group (Table 2).

Table 2: Evaluation of AST level in hypertensive patients taking atenolol alone

Sr. No.	Gender	Age	Duration of Administration	Dose (mg)	AST
1	Female	34	2 years 2 months	100	38
2	Female	35	1 week	50	66
3	Female	38	8 years	50	30
4	Female	38	1 years	50	24
5	Male	39	4 months	100	43
6	Female	42	1 year 1 month	50	60
7	Female	43	7 months	50	38
8	Female	43	7 years	100	41
9	Female	45	1 week	100	42
10	Male	45	9 months	100	32
11	Female	45	4 months	100	44
12	Male	47	6 months	25	43
13	Male	47	1 year	100	44
14	Female	47	1 year 3 months	100	50
15	Female	48	2 years 10 months	100	278

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16	Female	48	1 year 1 month	50	41
17	Male	48	7 months	25	28
18	Male	50	1 year 3 months	100	33
19	Male	50	1 year 1 month	50	55
20	Female	50	3 years	100	52
21	Female	50	1 year 2 months	50	38
22	Female	50	6 months	100	50
23	Female	50	5 months	50	51
24	Female	51	7 months	20	50
25	Female	52	9 months	100	57
26	Female	54	1 year 3 months	50	35
27	Male	56	2 years	50	35
28	Male	60	1 month	20	34
29	Male	60	1 month	25	37
30	Female	60	6 years 1 months	50	82
31	Female	60	10 months	100	56
32	Female	60	2 years 2 months	100	27
33	Female	60	1 year 9 months	50	36
34	Female	60	5 months	100	50
35	Female	60	7 years	50	42
36	Male	61	7 years	100	63
37	Female	62	4 months	100	36
38	Male	65	2 years 7 months	50	58
39	Female	67	6 months	50	38
40	Male	70	1 year	50	43
41	Female	70	5 months	100	36
42	Female	70	1 year 2 months	50	38
43	Male	77	2 weeks	100	53

While, in the group of patient's takings atenolol in combination with other antihypertensive drugs having random age and gender have lower disturbance in AST levels as compared to group of patients taking atenolol alone(Table 3).

Table: 3 Evaluation of AST level in hypertensive patients taking atenolol in combination

Sr. No.	Gender	Age	Duration of Administration	Dose (mg)	AST
1	Male	33	1 year	100	58
2	Female	35	8 years 2 months	100	35
3	Male	40	3years 3 months	100	29
4	Female	40	8 months	50	41
5	Male	41	1 year 3 months	100	53
6	Male	43	1 month	50	54
7	Male	44	5 years 4 months	50	36
8	Female	45	9 months	100	58
9	Male	45	5 months	50	56
10	Male	48	1 week	100	45
11	Female	48	4 years 5 months	50	28
12	Female	50	1 week	100	38
13	Male	50	6 years	100	44
14	Male	50	1 week	100	26
15	Female	50	1 year	50	60
16	Female	50	1 week	50	46
17	Female	54	3 years 3 months	100	60
18	Male	54	2 months	50	61
19	Female	55	9 years	50	63
20	Male	55	4 years 3 months	100	52
21	Male	56	1 year 1 month	100	29
22	Male	57	4 years 3 months	100	39
23	Male	58	1 year 5 months	50	32
24	Male	58	10 years	50	46

The results were further subjected to statistical analysis by applying standard deviation and standard error. In case of AST values evaluation, the significant results with 0.049 value were obtained(Table 4).

Table 4: Significance of measured AST values

values	N	Mean ± SD	Standard. Error	Significance
AST	.00	32.10 ± 8.69	1.94246	0.049
	1.00	49.46 ± 37.51	5.72064	
	2.00	42.24 ± 11.53	1.89689	
	Total	43.32 ± 26.49	2.64983	

DISCUSSION

Hypertension is a medical condition attributed to high blood pressure in blood vessels (140/90 mmHg or higher). According to world health organization ~1.28 billion adults with age between 30 to 79 years have hypertension across the world, with almost two-thirds of them living in low and middle economic countries. Some common factors include; age, genetics, obesity, non-active lifestyle, diet with high salt content and alcohol consumption [14]. Different anti-hypertensive drugs have developed to control blood pressure which targets different systems of the body that control and regulates the blood pressure. These anti-hypertensive drugs include; alpha blockers, ACE inhibitors, angiotensin receptor blockers, beta blockers, calcium channel blockers, central alpha agonists, diuretics, renin inhibitors and vasodilators [15]. Among beta blocker, a known drug is Atenolol (2-{4-[2-Hydroxy-3-(propan-2-ylamino) propoxy] phenyl} acetamide), which is a beta-adrenergic agent that blocks beta receptors on the heart and slow it down and decreases the blood pressure [9]. Some side effects of atenolol have been found when it is used in treatment of hypertension including; indigestion, dry mouth, depression, hepatic dysfunction, constipation, confusion, insulin level disturbance, central nervous system side effect, gastrointestinal and cardiovascular dysfunction [16]. Among these side effects the main focus of study is to study the effect of atenolol on hepatic dysfunction. As it is reported to cause damage to the liver cells. Liver function tests (LFTs) are important clinical assay used in clinical laboratory to give information about the condition of patient's liver [17]. Aspartate transaminase (AST) also known as serum glutamic oxaloacetic transaminase (SGOT) is an important biomarker among these LFTs but it doesn't indicate the absolute dysfunction of liver as its value can also be increase in some other inflammatory conditions such as muscle damage [13]. However, it is most commonly used test in clinical laboratories. In this study the effect of atenolol is studies on liver dysfunction by measuring AST level from blood of 80 patients either treated with atenolol alone or with atenolol in combination with other hypertensive drugs. While 20 persons with random age and gender with blood pressure within normal range were taken as control. After

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blood sampling the serum was separated and AST level was measured by measuring the change in absorbance with time due to the conversion of NADH to NAD+ which was measured by using photometer at 340/400nm rate technique. The significant results were obtained as there was more increase in level of AST in patients treated with atenolol alone (mean value = 49.9) as compared to patient group treated with atenolol in combination with other anti-hypertensive drugs (mean value = 42.2) and control (mean value = 32). The effect of atenolol on hepatic dysfunction has found in literature, as one study has reported that out of 76408 people having side effects due to atenolol, 393 (0.15%) have abnormal liver function tests. While in sixty the rate of hepatic dysfunction increases while using atenolol. In females this chance of hepatic dysfunction increases while using atenolol [12]. Many other studies have also reported the hepatic dysfunction due to atenolol treatment for hypertension as one study has reported that in two patient's hepatic dysfunction was reported and in another complicated case the liver cirrhosis (acute hepatitis) was reported on administration of atenolol in hypertensive patient [18, 19]. Result of this study supports these previous studies of effect of atenolol on hepatic dysfunction by evaluating the AST value [20, 21].

CONCLUSIONS

This study has showed that atenolol may have efficacy in controlling hypertension but it also causes disturbance in AST levels so any other drug in combination with atenolol is recommended to use to control hypertension to avoid AST variation. Further this study can be performed on larger patient groups with more cases for validating the outcomes of this study.

Authors Contribution

Conceptualization: MFS
Methodology: MR, IS
Formal analysis: IT, SK
Writing-review and editing: MFS, IS, IT, SK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Determination of Levels of Anxiety and its Association with Demographic Characteristics of Adolescent Girls in Secondary Schools of Lahore

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ABSTRACT

Adolescence is a transition period between the time of childhood and adulthood as it is usually accompanied by sexual maturation and physical growth as well as psychological and cognitive changes necessary for coping with the tasks of later adult life. **Objective:** To identify levels of anxiety and its association with demographic characteristics of adolescent girls in secondary schools of Lahore **Methods:** Cross-sectional, analytical design was used in this study. The normality assumptions were tested by Kolmogorov-Smirnov[a] and Shapiro-Wilk test. **Results:** Out of 140 participants, 116 participants were having anxiety. Among these participants, 73 (62.9%) were having mild anxiety, 38 (32.7%) were having moderate anxiety, and only 5 (4.31%) were having severe anxiety. Statistical analysis show that the data were not normally distributed as evident from the p-value 0.01 and 0.03 in Kolmogorov-Smirnov[a] and Shapiro-Wilk tests, respectively. Analysis showed that there is a significant difference in anxiety levels in adolescent school girls as evident by p-value is less than 0.05. **Conclusion:** The prevalence of anxiety was 82.85% among adolescents' girls. Majority of the adolescent students were facing mild and mild anxiety and this anxiety remain throughout the adolescent age but reduce with passage of age.

INTRODUCTION

Anxiety is a wide term that refers to a person's predisposition to feel uneasy, tense, or awkward in social interaction with strangers [1]. Due to changes in the body the anxiety increases among adolescent girls due to physical appearance of body which are increased with changes and are gradually reduced after full puberty [2]. However numerous examinations utilize menarche – the beginning of first menstrual cycle as an intermediary for beginning of pubescence in females, and relationship between early menarche and expanded levels of depressive side effects and misery in pre-adulthood are generally announced [3]. Pubertal changes affect the adolescent self-image, mood and interaction with peers and parents. Pubertal changes make them confused, unsafe and egocentric. Mental disorders such as anxiety, depression, eating disorder and fear of thoughts are common during anxiety [4]. Adolescence is regarded as a private affair or a family problem that must be handled. A variety of taboos exist that add to the strain placed on young females. Young girls have no clue how to cope with the issues that arise during this transition period, or where and how to obtain relevant information. Due to cultural norms, they are unable to freely share their difficulties and receive assistance. Only mothers can lead children with limited information, and the mother's own knowledge is also variable, so an illiterate mother can't properly guide

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them [5]. Young girls go through a number of changes before they reach adulthood. During this period, this age group encounters a variety of problems. One highlighted problem of adolescence is anxiety. An abnormal and overwhelming sense of apprehension and fear often marked by physical signs (such as tension, sweating, and increased pulse rate), by doubt concerning the reality and nature of the threat and by self-doubt about one's capacity to cope with it. The emotional state of a student has a significant impact on their overall performance, which has a range of repercussions on both an individual and professional level. Anxiety can present itself physically in a variety of ways. The cardiovascular, respiratory, nervous, and gastrointestinal frameworks are frequently associated with these symptoms [6]. Expanded circulatory strain, fast heart rate with palpitations and tachycardia, sweating, dry mouth, queasiness, discombobulating, hyperventilation, fretfulness, tremors, sleep deprivation, poor fixation, and feelings of shortcoming are the most common physiological manifestations of any circumstance specific uneasiness. Genuine concern is required for students who are experiencing extreme worry or despair. According to research, if they are not treated, they are more likely to perform poorly in school, have less developed social skills, and be powerless when it comes to drug usage. Female sexual orientation is more likely to experience anxiety [7]. Furthermore, a study shows that the onset of puberty and enlargement of breast and genetalia are associated with depression and varied in different gender, early onset of breast development is associated with high degree of depression in girls rather than in boys. Early onset of development of pubic hair unrelated with depression in both boys and girls [8]. Therefore, a relationship is assessed between adolescence and depression, yet numerous things remain ineffectively comprehended. While evaluating pubescence in females, most examinations consolidate pointers of breast and pubic hair improvement which are controlled by various hormonal pathways [9].

METHODS

Cross-sectional, analytical design was used in this study. The setting for this study was two government girls' secondary schools and two private girls' secondary schools of Lahore. The study was conducted in 9 months after the approval of synopsis. Sample size of 140 cases is calculated with expected mean score of anxiety among students i.e., 5.3±3.61 [10]. Simple Random sampling technique was used to collect the data. All the student girls studying in 9th and 10th grade in Government & Private Girls High Schools of Lahore, All the student girls age between 14-17 years, Students having menarche experience were included. Death of family member in last 6 months, suffer mental trauma last 6 months and Students who suffering from mental diseases was excluded from the study were excluded. Permission was taken from the Headmistress of Government & Private Secondary girls School of Lahore. Consent was be signed from each participant. The information of respondent was be kept confidential. The participants were having full authority to withdraw at any time. Data were collected from the participants at breaktime, every 3rd student from the class was selected to collect the data. Both Urdu and English Questionnaires were distributed, every participant was having the option to fill the questionnaire in Urdu or English. 30 Minutes given to fill the Questionnaire. Every questionnaire was consisted of six demographic related questions and 22 Questions were related to Anxiety. The Beck Inventory Anxiety scale shows the intensity of feelings: (0) Never (1) Very Less (2) Sometimes (3) Often, and (4) always in responding to the Beck Inventory scale shows that how they generally feel by rating the frequency of their feeling of anxiety by five-point scale (0) Never, (1) Very Less (2), Sometimes (3), Often, and (4) always. 22 questions were consisted of total 110 scores. The score of anxiety below 30 was termed as no anxiety, score 30-50 was considered as mild anxiety, score from 51-70 was termed as moderate anxiety and score above 70 was considered as severe anxiety. Data were analyzed in SPSS software version 21. Descriptive analysis was performed on continuous variables data whereas frequencies and percentage were used for categorical variables. Furthermore, to check the normality Kolmogorov-Smirnov[a] and Shapiro-Wilk tests were used. Based on that the Mann-Whitney U test was used to check the difference in anxiety among adolescent female students of government and private schools. Whereas, Chi-Square Test was used to identify the associations among levels of Anxiety and demographics characteristics of adolescent school going female students. *p*-Value of Less than 0.05 were considered statistically significant.

RESULTS

Demographic results were checked by applying the Frequencies and Percentages on SPSS Version 21. Mean age was 15.2 years. Age of the participants 14 years were 50 (35.7%), 15 years were 34 (24.3%), 16 years were 31 (22.1), and 17 years were 25 (17.9%)., In the education of the father were mostly illiterate 55 (39.3%), primary pass was 31 (22.1%), matriculation education were 52 (37.1%), and graduates were only 2 (1.4%). In the education of the mother were mostly were illiterate 84 (60%), primary pass was 25 (17.9%), matriculation education was 27 (19.3%), and graduates were only 4 (2.9%). Occupation of the participants' father's own business were 51(36.4%). labors

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were 42 (30%), jobs were 26 (18.6%), and 21 (15%) were surprisingly unemployed, Occupation of the participants' mother, majority of mothers were housewife 123 (87.9%), labors were 8 (5.7%), jobs were 6 (4.3%), own business were only 3 (2.1%), Number of family members between 6-8 were 63 (45%), 9-11 were 44 (31.42), 3-5 were 24 (17.1%), and 12-14 were 9 (6.4%) as shown in Table 1.

Table 1: Demographic characteristics of participants

	Categories	Frequencies (%)
Age of the Participants	14 Years	50(37.5)
	15 Years	34(24.3)
	16 Years	31(22.1)
	17 Years	25(17.9)
	Total	140(100)
Education of the Father	Illiterate	55(39.3)
	Primary	31(22.1)
	Matric	52(37.1)
	Graduation	2(1.4)
	Total	140(100)
Education of the Mother	Illiterate	84(60)
	Primary	25(17.9)
	Matric	27(19.3)
	Graduation	4(2.9)
	Total	140(100)
Occupation of Father	Unemployed	21(15)
	Labor	42(30)
	Job	26(18.6)
	Own Business	51(36.4)
	Total	140(100)
Occupation of Mother	Housewife	123(87.9)
	Labor	8(5.7)
	Job	6(4.3)
	Own Business	3(2.1)
	Total	140(100)
No. of Family Members	3-5	24(24)
	6-8	64(45.7)
	9-11	43(30.7)
	12-14	9(6.4)
	Total	140(100)

The prevalence of anxiety was 82.9% (116) in the studied sample whereas 24 (17.1%) were having no anxiety. Out of 116 participants, 73 (62.9%) were having mild anxiety, 38 (32.7%) were having moderate anxiety, and interestingly only 5 (4.31%) were having severe anxiety as shown in above table. The normality assumptions were tested by Kolmogorov-Smirnov[a] and Shapiro-Wilk test. The analysis show that the data were not normally distributed as evident by the p-value 0.01 and 0.03 in Kolmogorov-Smirnov[a] and Shapiro-Wilk tests, respectively (Table 2).

Table 2: Prevalence of Anxiety and levels of Anxiety

Level of Anxiety among Adolescent Girls		Prevalence
Anxiety Level	f (%)	116/140X100= 82.85%
No Anxiety	24 (17.1%)	
Mild Anxiety	73 (52.1%)	
Moderate Anxiety	38 (27.1%)	
Severe Anxiety	5 (3.6%)	
Total	140 (100%)	

Table 3 shows difference in anxiety among government and private adolescent girls. Mean rank of Public schools was 79.39 and mean rank of private schools was 61.61.

Table 3: Difference in Anxiety among Government and Private Adolescent Girls

	Group Public Private	N	Mean Rank
Anxiety Scores	Public Schools	70	79.39
	Private Schools	70	61.61
	p-Value		.010

p-Value is calculated by *Mann Witney U Test*

Chi-Square Test was applied, *p*-value was 0.52, and hence, results were not statistically significant according to the age of the participants. Adolescent girls having age 14 years were 50 (37.5%), out of these, anxiety was present in 42 participants. Moderate anxiety was present in 25 (59.5%), mild anxiety in 15 (35.7%) and severe anxiety in only 2 (4.76%). Girls with age 15 years were 34 (24.3%), out of these, 27 were having anxiety. Moderate anxiety was observed in 14/27 (51.9%), mild in 12/27 (44.44%) and severe in only 1 (3.7%). Among 16 years old girls 31 (22.9%), 24 were having anxiety. Out of 24 participants, 15 (62.5%), were having mild anxiety, 7 (29.1%) were having moderate anxiety and only 2 (8.33%) were having severe anxiety. 25 (17.1%) girls were 17 years old girls, out of 23 Adolescent girls were having anxiety. Out of 23 participants, 21 (91.3%), were having mild anxiety, 2 (8.7%) were having moderate anxiety.

Table 4: Level of Anxiety according to Age

Level of Anxiety According to Age					
Age	No Anxiety	Mild Anxiety	Moderate Anxiety	Severe Anxiety	Total f (%)
14 Years	8	25	15	2	50 (37.5%)
15 Years	7	12	14	1	34 (24.3%)
16 Years	7	15	7	2	31 (22.1%)
17 Years	2	21	2	0	25 (17.9%)
Total	24 (17.2%)	73 (52.2%)	38 (27.1%)	5 (3.5%)	140 (100%)

DISCUSSION

In this research out of 140 participants, most of the participants (73, 52.2%) had mild anxiety, 38 (27.1%) were facing moderate anxiety and 24 (17.2%) had no anxiety. A similar study was conducted at high schools in Qasim regions of Saudi Arabia in 2018 in which it was found that out of 1245 participants, 36% were not facing anxiety, 34.1% were having mild anxiety, 29.5% moderate anxiety and 9.8% were facing severe anxiety [11]. A Study was conducted in Rawalpindi, Pakistan in which 21.4%

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adolescents were having the anxiety. This study also revealed that anxiety and well-being were significantly associated with the adolescent's age. Lower level of education, deprived socioeconomic status are also associated with higher level of anxiety in Pakistani adolescents [12]. According to the age of the participants, the mild level of anxiety remained throughout the adolescent age, 25 (17.9%) participants of 14 years, 12 (35.2%) of 15 years, 15 (48.4%) of 16 years and 21 (84%) of 17 years age. Moderate level of anxiety reduced with the passage of age. A study was conducted in Rawalpindi in 2019 and observed anxiety throughout the adolescent age 11-18 years [12]. One more study was conducted in Italy, 75% adolescents were facing anxiety [13]. It shows that most of the girls face mild and moderate level of anxiety throughout the age of adolescent. Participants in a prior study varied in age from 15 to 18 years old and had a mean age of 16.39±14.339 years. A prior research found that the majority of higher secondary school pupils (52 %) were between the ages of 15 and 18 years [14]. According to the current findings, out of 24 (17.1%) individuals, 73 (52.1%) had mild anxiety, 38 (27.1%) had moderate anxiety, and 5 (3.5%) had severe anxiety. A prior research found similar results, with 30.4% of participants reporting moderate anxiety, 20.7% reporting moderate to severe anxiety and 21% reporting severe anxiety [15]. Another research published in China had similar findings, revealing that 63.8% of teenagers go through puberty without receiving any psychological support [16]. In a previous study with 400 post-menarche females between the ages of 14 and 20 years, anxiety scores were found higher in females with dysmenorrhea. It is thought that dysmenorrhea disrupts healthy physical and mental development in adolescence by impairing the quality of life with increased days of absence from school [17]. The results of a previous study revealed that the frequency and percentage distribution of level of anxiety among the school going adolescents. In school going adolescents, majority of them had moderate level of anxiety 22 (73.3%), 6 (20%) had mild level of anxiety and few school going adolescents had severe level of anxiety 2 (6.7%) [18]. Teenagers' levels of depression and anxiety can be influenced by a variety of risk and protective factors (individual, familial, and social) within an ecological framework [19, 20].

CONCLUSIONS

The prevalence of anxiety was 82.85% among adolescents' girls. Majority of the adolescent students were facing mild and mild anxiety and this anxiety remain throughout the adolescent age but reduce with passage of age.

Authors Contribution

Conceptualization: MA, MA
Methodology: ZI, MA
Formal analysis: SR
Writing-review and editing: MA, SR

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

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Original Article

Knowledge, Attitude and Practice towards Exclusive Breastfeeding among Lactating Mothers visiting Sir Ganga Ram Hospital, Lahore: A Descriptive Cross-Sectional Study

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ABSTRACT

Breastfeeding is important public health issues having numerous benefits for both infants and mothers but rates of exclusive breastfeeding remain low throughout the world. Several studies have investigated the factors that influence breastfeeding behaviors including maternal education, social support, and cultural beliefs. However, there is still much to be learned about the knowledge, attitudes, and practices of lactating mothers and how these factors affect breastfeeding outcomes. **Objective:** To assess knowledge, attitude, and practices of exclusively breastfeeding among lactating women attending Sir Ganga Ram Hospital. **Methods:** The cross-sectional study is carried out in Sir Ganga Ram Hospital, Lahore. The study is conducted in 4 months from February 2023 to May 2023. SPSS version 21.0 is used for data analysis. In this research, all individuals aged from 18 to 40 years; mothers having infant of from 0-24 months from Pediatric, Gynecology Wards and OPD were included. In exclusion criteria: non-cooperative mothers. Mothers with psychological problems were not bothered. Mothers with severely ill infants (e.g., cleft palate) **Results:** 54% of mothers were within normal ranges of BMI and only 4% were within the range of obesity. In our study, 46% of lactating mothers had 1-2 children and 13% had 5 or more than 5 children. 67% of mothers believed breastfeeding helped gain optimal weight gain.44% of mothers gave their child colostrums. About 28 Children <4 months were breastfed less than 4 times. **Conclusion:** The study shows that mothers were well informed and had proper knowledge, positive attitude and behavior towards breastfeeding. Most of the lactating mothers know the benefits of breastfeeding for 2 years.

INTRODUCTION

Human milk or breast milk is an ideal food for newborn babies and infants [1]. As breastfeeding is beneficial for infants, it is also beneficial for mothers. It decreases the risk of breast and ovarian cancer in females [2]. There are significant benefits for the babies that were fed on breast milk for the first two years after birth [3]. Breast milk is a unique combination of all the vital nutrients and antioxidants an infant's body needs to grow mentally and physically [4]. WHO has recommended that breastfeeding should start in the first hour of birth and continue till 6 months without any other food or liquid. Weaning should start at the age of 6 months but breastfeeding should not be discontinued until the age of 24 months. Children who did not receive complementary meals on time had a greater risk of wasting. Infants who did not obtain the required amount of nutritional diversity had a greater risk of being underweight than those who did. In comparison to their counterparts who received the minimal feeding frequency,

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children who did not receive it were more likely to be stunted. Hence it is proved that infant malnutrition is predisposed by inadequate complementary feeding [5]. According to several studies, the attitude of a mother towards breastfeeding is very important for initiation, duration and decision regarding breastfeeding [6]. So, it has been found that to raise the rate of exclusive breastfeeding, it is vital to boost education before and after birth, refrain from using any other nutrition, pacifiers, or bottles after delivery, and spend appropriate time with the infant [7]. The knowledge about breastfeeding is very important as if a mother has proper knowledge about breastfeeding, it will help her in the process and same goes with the attitude of a mother towards breastfeeding and the health of her child. Improved Knowledge and attitude could contribute in increasing the prevalence of exclusive breastfeeding in both working and non-working mother [8]. Regular continuous individualized prenatal education and postnatal assistance can effectively enhance rates of exclusive breastfeeding from birth to 4 months postpartum and modify breastfeeding behavior [9]. Exclusive Breastfeeding was predicted by age, anticipated pregnancy, postpartum education, frequent nighttime nursing, and formula introduction time. Pregnant adolescents may begin nursing sooner if the pregnancy is planned and the family and medical professionals educate and promote breastfeeding. As a result, methods for improving breastfeeding programs should be developed [10]. Undoubtedly, breastfeeding exclusively is a crucial infant feeding method that helps ensure children's health and wellness, particularly in developing and underdeveloped nations. This feeding technique not only ensures a child's survival but also benefits the mother's health and offers defense against several non-communicable illnesses [11]. Even though many mothers were aware of the value of colostrum, the statistics show that more work must be done to change people's knowledge, attitudes, and practices towards colostrum feeding [12]. The world's exclusive breastfeeding rate is now at 38%, but the World Health Assembly set a goal in 2012 to raise it to at least 50% by 2025 [8]. Despite these guidelines, it has been shown throughout the time that the practice of exclusive breastfeeding has not been widely embraced; most women support the notion but do not exclusively nurse their babies for the first few weeks after giving birth. Numerous aspects, including cultural, societal, and economic circumstances, have been noted as potential barriers to the effective practice of exclusive breastfeeding [13]**.** The BSP proved to be an efficient technique for delaying any breastfeeding cessation, hence increasing breastfeeding length and exclusivity. Also, this intervention could help promote breastfeeding if implemented widely [14]. Health education programs and neonatal feeding strategies are affected by late breast-feeding initiation [15]. More noteworthy public mindfulness, regulations that support breastfeeding in broad daylight and the work environment, as well as help of relatives may all be critical to effective breastfeeding [16]. To improve exclusive breastfeeding practice, it was suggested that women be educated, husbands be engaged, antenatal care follow-up be encouraged, and exclusive breastfeeding counseling be provided during antenatal care [17].

METHODS

The cross-sectional study is carried out in Sir Ganga Ram Hospital, Lahore. The study is conducted in 4 months from February 2023 to May 2023. SPSS version 21.0 is used for data analysis. In this research, all individuals aged from 18 to 40 years; mothers having infant of from 0-24 months from Pediatric, Gynecology Wards and OPD were included. In exclusion criteria: non-cooperative mothers. Mothers with psychological problems were not bothered. Mothers with severely ill infants (e.g., cleft palate).

RESULTS

Table 1 demonstrates the demographic characteristics of the participants; number of children, BMI of mother, Maternal Employment status, General Health status of mother, education level of mothers. In the above table majority (54%) of mothers are at normal BMI and about 28% are underweight., whereas most of the mothers below to age of 21-25 years. About 75% having C-section delivery, most of the mothers have 1-2 numbers of children. 54% below to urban area and 42% are Illiterate and 39% are graduate or above. 68% are housewives, 46% mothers have good health status.

Table 1: Demographic Characteristics of Participants

Number of Children	Frequency
1-2	46
3-4	40
5 or more	13
Type Of Delivery	**Frequency**
Normal	43
C-section	57
BMI Of Mother	**Frequency**
<18.5	28
18.5-24.92	54
5-29.9	14
30-34.9	4
Family Background	**Frequency**
Rural	44
Urban	56
Maternal Education Level	**Frequency**
Illiterate	42
Intermediate	19
Graduate or above	39

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Maternal Employment Status	Frequency
Housewife	68
Service	32
Socioeconomic status	**Frequency**
A	26
B	54
C	20
General Health Status Of Mother	**Frequency**
Healthy	30
Good	46
Poor	24

Table 2 demonstrates the importance of breastfeeding, age gap between children, benefits of breastfeeding, initiation of breastfeeding, effect of bonding between mother and child, benefits of formula milk and if anyway breastfeeding can harm your child.

Table 2: Knowledge of lactating mothers towards exclusive breastfeeding

Do you know the importance of Breast feeding?	**Frequency**			
	Yes		**No**	
	91		9	
Do you think Breastfeeding can harm your child in anyway?	**Frequency**			
	Yes		**No**	
	16		84	
Do you think formula milk can be beneficial to your child	**Frequency**			
	1 year	**2 year**	**3 year**	**4 year**
	31	41	23	5
Do you think breastfeeding is beneficial for mother?	**Frequency**			
	Yes		**No**	
	85		14	
At what time did you initiate breastfeeding to your child after delivery?	**Frequency**			
	1 hr. of birth	**Between 1-4hrs**	**24hrs of birth**	**2nd day onwards**
	27	31	17	25
Is there any effect of breast feeding between the mother and child bond?	**Frequency**			
	Bonding increase		**No effect**	**Bonding decrease**
	86		14	0

Table 3 demonstrates the changes in the body due to breastfeeding, nutritive value of substitute formula milk is same as the mother milk, extra food than breast milk and is it necessary to give water along with breast milk and if fenugreek and almonds can improve production of milk.

Table 3: Attitude of lactating mothers towards exclusive breastfeeding

Can fenugreek and almonds improve milk production?	**Frequency**	
	Yes	**No**
	53	47
Is it necessary to give water along with breast milk?	**Frequency**	
	Yes	**No**
	23	77
Can breastfeeding bring any changes in the body shape?	**Frequency**	
	Yes	**No**
	80	20
The nutritive value of substitute formula milk is same as the mother milk?	**Frequency**	
	Yes	**No**
	48	52
Do you feel comfortable giving extra food rather than breast milk to your infant?	**Frequency**	
	Yes	**No**
	56	44

Table 4 demonstrates the questions regarding practices of exclusively breastfeeding; demand of breastfeeding, colostrums feeding, breastfeeding during illness of child and your illness, first nutrient to your baby.

Table 4: Practices of lactating mothers towards exclusive breastfeeding

Do you breastfeed on demand?	**Frequency**		
	Yes		**No**
	74		26
How frequently do you feed your child during the night?	**Frequency**		
	1-3 times		**More than 3 times**
	47		53
Did you give colostrum to your baby?	**Frequency**		
	Yes		**No**
	44		56
Does excusive Breastfeeding help the child to gain optimal weight?	**Frequency**		
	Yes	**No**	**Don't Know**
	57	33	10
If you use formula milk do you know the exact method to make the formula?	**Frequency**		
	Yes		**No**
	92		8
Do you continue breastfeeding during your illness?	**Frequency**		
	Yes		**No**
	68		32
Do you continue breastfeeding if your child is sick?	**Frequency**		
	Yes		**No**
	74		26
What was the first nutrient given to the baby?	**Frequency**		
	Honey	**Plain water**	**Breast milk**
	77	1	22

DISCUSSION

The research was conducted to study the knowledge, attitude and practices of exclusive breastfeeding in lactating mothers by visiting Sir Ganga Ram Hospital, lactating mothers were selected through non-probability convenient sampling technique. According to the results, 46% of lactating had 1-2 children, 40% lactating mothers had 3-4 children while 13% had 5 or more children. In addition, the socioeconomic status of lactating mothers and their family also contribute, 26% mothers belong to upper class, 54% belongs to middle class and 20% belongs to poor class category. Moreover, the health status of a mother is also associated with the health of an infant. The results have shown that 30% of mothers are healthy, 46% are in good status of health while 24% of mothers had a poor health status. Furthermore, the maternal employment status also effects the continuation of

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breastfeeding till optimal age. The results have shown that 68% lactating mothers are housewife and 32% of them are working women. The maternal education of mothers also had a huge impact on the knowledge, attitude and practices of lactating mothers towards exclusive breastfeeding. the illiteracy level of lactating mothers is 42% while 19% of mothers has intermediate level education and 39% are graduated. Similarly, a survey was conducted and published by John Elflein in 2017. The results showed that majority 90% of mothers were graduated and they initiated breastfeeding and continued it while 69% had less education or diplomas [18]. Current studies have shown that most of the lactating mothers have proper knowledge, a positive attitude towards breastfeeding and they practice breastfeeding till 2 years after the birth. The study is conducted among 100 lactating mothers out of which 56% mothers considered colostrum is good for baby's health while 38% mothers had no idea what colostrum is and 6% mothers do not consider it beneficial. While 68% mothers also believe that it is fine to continue breastfeeding during their illness and 32% mothers do not feed their infant when they are not feeling well. Similarly, a cross-sectional study was conducted by Bashir *et al.*, in 2018 [19]. By using interviews and semi structured questionnaire, data were gathered. In this study conducted among 100 lactating mothers we have also concluded that 83% of mothers stated that they have practiced breastfeeding after the birth of their child while 17% did not practice breastfeeding and they believe formula milk could fulfill the nutritional needs of their child. Similar results were found in a cross-sectional study was conducted by Ayalew in 2016 [20]. It is a community-based study. The purpose of this cross-sectional study was to determine the prevalence and factors among first-time mothers about exclusive breastfeeding. Results shows that 57.3% of women out of 400 used exclusive breastfeeding 24 hours before the survey. Compared to the nationally advised threshold, a significant number of moms practiced exclusive breastfeeding at a low level. These findings suggest that in order to increase exclusive breastfeeding among first-time mothers, multidisciplinary and intersectoral strategies are required [19]. This study also revealed that among 100 lactating mother, 65% mothers have proper knowledge about breastfeeding and only 35% mothers have very less or no knowledge about breastfeeding. In this survey we have concluded that 65% mothers breast feed their child for first 6 months while 35% mothers did not give breast milk to their infant for 6 months after birth. Similar type of study was conducted by the authors of this cross-sectional study was Hegazi *et al.*, and this study was conducted in 2019 [21]. This study was conducted to find out the prevalence of Exclusive breastfeeding and variables influencing this type of breastfeeding. The data were collected from 420 breastfeeding mothers. The results of this study indicate that six-month prevalence of Exclusive breast feeding was 27.6%, which is significantly lower than what the World Health Organization recommends [20]. According to this study we have concluded, 68% mothers consider formula milk beneficial to the health of their infant while 32% of lactating mothers do not think that giving formula milk instead of breast milk is beneficial for the baby's health. In another factor, lactating mothers were asked the time of initiation of breastfeeding after the birth. Among 100 lactating mothers, 27% start breastfeeding within the 1st hour after delivery, 31% of them initiated breastfeeding within 1-4 hours after delivery, 17% of mothers fed their child breastmilk within 24 hours while only 25% of mothers initiated breastfeeding from 2nd day onwards. A similar study took place by Yilmaz *et al.*, in 2016 [10]. In present research, early breastfeeding initiation, the prevalence of Exclusive Breastfeeding throughout the first six months. 200 adolescent mothers having children aged 6 to 24 months participated in this cross-sectional research. The proportion of patients who began breastfeeding within one hour was 45.5%. As the baby's first source of nourishment, 74% of adolescent moms chose nursing over any sort of formula [10]. The current study has shown that duration of breastfeeding and the health of the baby is very much dependent on the employment status of the mother. In the results, data collected from 100 lactating mothers, 40% mothers were housewife and considered that breastfeeding is the best way to feed the child while 28% of them think that formula milk is also a good option. On the other hand, 15% working mothers consider formula milk is good for the health of the infant and it is easily available while 17% working mothers do not give formula milk to their child. From another similar cross-sectional study, carried out by Salcan *et al.*, in 2016 [7]. The study is done among 100 lactating mothers out of which 86% mothers stated that bonding increases between mother and child while 17% mothers think that breastfeeding has no effect of any type on mother-child bonding. A similar study was conducted by the authors Hongo *et al.*, in 2015 [23]. In this study, the relationship between breastfeeding satisfaction, length of the breastfeeding, and exclusivity among mothers and baby-friendly breastfeeding support was described. Results shows that early skin-to- skin contact with newborns increased the pleasure of breastfeeding among nursing mothers compared to mothers who did not do skin to skin contact. When compared to mothers who did not intend to breastfeed exclusively, those who were encouraged to do so were more likely to breastfeed exclusively at one month and to believe that breastfeeding is beneficial to their child [17]. The current study has shown

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the positive attitude and practices towards breastfeeding. The frequency of night time breastfeeding is calculated through given questionnaire to lactating mothers. The results are that 53% of lactating mother breastfeed their child more than 3 times at night while 47% mothers give feed to their child 1-3 times at night. The authors of this cross- sectional study are Carillo-Diaz *et al.*, and this research was conducted in 2021. This study was conducted to evaluate the relationship between early childhood carries (ECC) and nighttime breastfeeding. 212 children (aged 2-4 years) who breastfed at night was evaluated. Less than 18 months of breastfeeding resulted in a lower dft index for the group (p = 0.02). Furthermore, there were significant differences in the dft index between co-sleepers for eighteen months or more and those who co-slept for less than eighteen months (p 0.05), as well as between co-sleepers for eighteen months or more and those who did not co-sleep (p 0.01). Hence, it is proved that nighttime nursing after 18 months is thought to increase the likelihood of early childhood carries [24]. From the current study we have derived the knowledge, attitude and behavior of lactating mothers towards colostrum feeding. Among 100 lactating mothers, 56% mothers consider colostrum is good for infant while 6% mothers think it is bad for the health of infant and 38% have no idea what colostrum is and how it is important for the newborn. Another question that was asked to lactating mothers is if they see any counsellor or health care provider if they face any difficulty during breastfeeding, 19% mothers said that they consult to health care provider if they face any issue regarding breastfeeding while 81% mothers do not consult to any health care professional. The cross-sectional study was conducted by Aisha R *et al.*, in 2014 [12]. The main goals of this research are to assess the understanding of pregnant women about the necessity of colostrum feeding and to encourage the practice of colostrum feeding. This research revealed that 35% of women learned about colostrum during prenatal visits, whereas 65% of women learned about them from the healthcare provider. Only 6% of moms were aware that the milk is beneficial for newborns. Only 9% women were aware of its protective qualities and knew that it aids in improving fetal development and infection prevention. While 35% of women believed that there is something toxic that is not good for the infant, 25% of women requested that this is the milk that should be fed to the baby, and 15% of women asked that this is the milk that should be thrown before feeding. Only 14% of the women in this survey recognized that the ideal time for feeding colostrum is shortly after delivery (1/2-1 hour), but 86% of women began feeding after 6 - 24 hours. 39% of the women had no knowledge about colostrum [12]. The current study has shown that most of the lactating mothers believe that exclusive breastfeeding helps in gaining optimal weight. The results have concluded from 100 lactating mothers out of which 57% lactating mothers think that exclusive breastfeeding helps child in gaining optimal weight, 33% lactating mothers do not believe that exclusive breastfeeding has any role in child's development or gaining optimal weight while 10% mothers do not have any knowledge about the link between exclusive breastfeeding and optimal weight. This study was conducted by Rito *et al.*, 2015 and its purpose was to find out the relationship between infant obesity and early life characteristics like breastfeeding, exclusive breastfeeding, and birth weight. Results shows that obesity prevalence rates were highest in Spain (17.7%), 2nd highest in Malta (17.2%), and then in Italy (16.8%). The percentage of infants who were exclusively breastfed for 6 months was greatest in Tajikistan (94.4%). In comparison to children who had been nursed for at least six months, the pooled study revealed that children who had never been breastfed or who had only received general or exclusive breastfeeding had increased probabilities of being fat [25].

CONCLUSIONS

Exclusive breastfeeding is an essential practice that provides optimal nutrition and protection to infants. It is essential to understand the knowledge, attitude, and practices of lactating mothers towards exclusive breastfeeding. The study found that mothers had a positive attitude towards exclusive breastfeeding and had good knowledge about its benefits. Significant number of lactating mothers had knowledge of breastfeeding and its importance while insignificant number of mothers thinks that formula milk could be beneficial to their infant. Most of the mothers exclusively breastfed their infants for 6 months while insignificant number of mothers exclusively breastfeed their infant for less than 4 months.

Authors Contribution

Conceptualization: YW, BR HMJ
Methodology: HMJ, MI, AR, JS
Formal Analysis: MI, AR, JS
Writing-review and editing: YW, BR, EF, WAZ, ATC

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

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Original Article

Non-Obstructive Coronary Artery Disease due to COVID-19 Infection: St-Segment Elevation due to Endothelial Dysfunction

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ABSTRACT

The ongoing inflammatory process in COVID infection is reported to cause acute cardiac injury either due to direct cytotoxicity or oxidative stress injury to myocytes. Hypercoagulability and endothelial dysfunction are the two main events resulting in the transient thrombotic process. **Objective:** To assess the coronary artery occlusion in COVID infected patients presenting with ST-elevation myocardial injury. **Methods:** A descriptive case series study was conducted on 52 patients presenting with ST-elevation myocardial infarction along with COVID infection. Data was collected regarding demographic profile, Troponin levels, COVID PCR, changes in ECG, Echocardiographic findings and coronary arteries were assessed angiographically. Data were analyzed using SPSS software and various percentages were calculated. **Results:** Of all the 52 patients studied, 46 patients (88%) tested positive for COVID -19 on RT-PCR done via nasopharyngeal swab. Other 6 patients tested negative initially but they had findings on their CXR and HRCT suggestive of interstitial pneumonia. All the patients were in age group 30-60 years with 32 patients (62%) being males and 20 being females (38%). None of the patients previously had any history of angina or myocardial infarction. Inflammatory markers were raised in about 43 patients (82.7%). ECG showed ST segment elevation in anterior leads in 35 patients (67%) and in inferior leads in 9 patients (17%) and in lateral leads in 2 patients (3.8%). Qualitative Troponin I test was positive in all the patients, whereas, about 41 patients (78%) had Quantitative Troponin I test in high probability range. About 50 patients (96%) had Ejection Fraction greater than 45% and normal segmental wall motion analysis. The coronary arteries were reported to be normal or recanalized in 40 patients (76%) and mild-moderate disease in single or two vessels are present in rest of the 12 patients. None of the patients had shown culprit artery severe disease or clot burden on angiograms. **Conclusions:** Acute Cardiac injury in previously asymptomatic patients may be related to the pro-thrombotic state created by COVID-19 infection resulting in endothelial dysfunction and mimicking ST-elevation myocardial injury raising Troponin levels indicated by raised D-Dimers and CRP level. The auto-recanalization of the coronary arteries and normal echocardiographic findings, are good prognostic factors for the post cardiac injury rehabilitation.

INTRODUCTION

COVID-19 virus, since its outbreak in Wuhan China, has taken many discourses in its presentation as viral pneumonia, affecting the gastrointestinal, nervous, cardiovascular systems and creating a hypercoagulable state in the body, raising D-Dimers and acute phase proteins. This novel disease has not only affected the patients with pre-existing cardiovascular disease but also those who had no previous presentation or risk factors (e.g. diabetes, hypertension, hyperlipidemia) [1]. It affected the first case in Pakistan on 26th Feb, 2020 raising the count to 692,231 confirmed cases locally till 05th April 2021, with second highest number of confirmed cases (233,348) in Punjab [2]. The signs and symptoms have changed with each passing phase spanning from simple flu, headache,

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and sore throat, high grade fever to dizziness, encephalopathy, diarrhea and interstitial lung disease. Many studies have reported the ongoing inflammatory process in COVID-19 infection causing acute cardiac injury either due to direct cytotoxicity or oxidative stress injury to myocytes. The elevated levels of Troponin I have been reported in about 19.7% COVID-19 patients by Shi *et al.*, among 416 hospitalized patients with mortality rate of 51.2% compared to 4.5% in those who showed no evidence of myocardial injury [3]. The major causes of raised troponin levels have been reported to be hypotension, microthrombi, cytotoxic insult, vasospasm and raised level of inflammatory markers [2, 3]. A New York based case series has reported 18 COVID-19 patients with non-obstructive coronary arteries on angiography with new onset ST-segment elevation myocardial injury [2]. A case report showing diffuse ST-elevation on ECG has been reported by Inciardi *et al.*, in which elevated troponin and Pro-BNP levels were found with normal chest X-ray and no signs and symptoms of interstitial pneumonia [4]. A study done by Chinese authors, Chen C. et al. shows cardiac manifestation of arrythmia, myocarditis, heart failure and pericardial effusion observed in a number of patients with a poor survival prognosis [5]. Xu X. *et al.*, reported development of cardiomyopathy in a cohort of 41 severely sick patients with signs and symptoms of COVID-19 infection and heart failure [6]. ICU admitted patients with normal electrolytes and tissue injury induced arrythmia (44.5%) have been retrospectively assessed and reported by Wang C. *et al.*, [7]. Alteration of the Angiotensin-converting enzyme 2 pathways have been implicated in causing direct myocardial injury too [2, 3]. Kamil B. *et al.*, reported a case, in which COVID-19 infection was shown to mimic the STEMI as there was no obstructed lesion on coronary angiography of the patient, whereas ECG showed ST segment elevation in inferior leads. Upon taking complete history, patient revealed influenza-like symptoms in the entire family, 2 weeks back and tested herself positive for CCOVI-19 PCR later [8]. Two case reports of Takotsubo cardiomyopathy and spontaneous coronary artery dissection have also been reported in COVID-19 patients [2, 3]. Mustafa S. *et al.*, reported a case of myocarditis in COVID-19 patient showing ST elevation changes in anterior leads with raised troponin levels and diffuse hypokinesia of Echo [9]. Thrombotic process ongoing in this infection is also reported to cause idiopathic central vein occlusion and left ovarian thrombosis apart from cardiological manifestations [2, 3]. Mayo Hospital Lahore, being a referral center for COVID-19 infected patients, enabled us to come across unusual and atypical cardiovascular findings in them. This study was mainly focused on reviewing the endothelial dysfunction in the coronary arteries in our local society, caused by inflammatory flare and hypercoagulability in COVID-19 infection affecting the patients with no previous cardiac history, presenting in the form of ST-segment changes and cardiac enzymes elevation with minimum abnormal findings on echocardiography and coronary angiography, as evidenced by a number of case reports and studies available internationally.

METHODS

A descriptive case series study was conducted by enrolling about 52 patients presenting to Cardiology department, Mayo Hospital, Lahore, referred from various medical wards for ECG changes i.e. ST-segment elevation in anterior, inferior or lateral leads, from June, 2020 to October, 2020. These patients were tested for COVID-19 by RT-PCR done on nasopharyngeal swab and initial presentation was mostly due to fever, cough, sore throat dyspnea and chest pain. All the patients shifted to Cardiology department underwent quantitative Troponin test, echocardiography and coronary angiography. The angiographic images were assessed for categorization of any coronary artery lesion and wall motion along with Ejection Fraction analysis, done on echocardiography. The findings were reported by well qualified consultants in the department. The detailed medical history of the patients was taken after informed consent, using a pre-tested questionnaire. CXR, ECG, D-dimers, CRP, HBA1c and other basic blood reports were retrieved after proper consent. Data was analyzed and stratified according to age, risk factors like hypertension, diabetes, duration of symptoms, findings on Echo and angiography. Percentages were calculated for different sub-groups using SPSS 21 software.

RESULTS

Of all the 52 patients studied, 46 patients (88%) tested positive for COVID -19 on RT-PCR done via nasopharyngeal swab. Other 6 patients tested negative initially but they had findings on their CXR and HRCT suggestive of interstitial pneumonia. Most of the patients (38 out of 46) were shifted to Cardiology department within 72 hours of their initial presentation. The 6 patients who tested negative but had typical high-risk findings were shifted after a stay of about 7-8 days in COVID-19 ward or ICU (Table 1 and Figure 1).

Table 1: Time of Shifting to Cardiology Department

Total enrolled Patients = 52		
Time of shifting to Cardiology department	**RT-PCR +ve for COVID-19**	**RT-PCR -ve for COVID-19**
Within 72hrs	38	-
After 72 hrs	8	-
At 7th-8th day	-	6

All the patients were in age group 30-60 years with 32 males (62%) and 20 females (38%). None of the patients

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previously had any history of angina or myocardial infarction. Diabetes was reported in 31 patients (59%) and HBA1c levels were correlated. Only 12 patients (23%) had history of hypertension and 31% had diabetes previously (Figure 2). Atypical, mild to moderate chest pain was present in only 20 (38%) of the total patients. Other symptoms reported were high grade fever in 4 patients (7%), low grade fever of 99-100°F in 24 patients (46%), grade II-III dyspnea in 32 patients (61.5%) and cough in 37 patients (71%) (Figure 3). Inflammatory markers like D-dimers, CRP and Ferritin levels were raised in about 43 patients (82.7%). ECG showed ST segment elevation in anterior leads in 35 patients (67%), in inferior leads in 9 patients (17%), in lateral leads in 2 patients (3.8%) and T inversions with non-specific ST-T changes in 6 patients (11%). Qualitative Troponin I test was positive in all the patients, whereas, about 41 patients (78%) had Quantitative Troponin I test in high probability range and 11 patients (21%) had the quantitative values in borderline range. In Echocardiography 50 patients (96%) had Ejection Fraction greater than 45% with normal segmental wall motion analysis. The coronary arteries were reported to be normal or recanalized in 40 patients (76%) and mild-moderate disease in single or two vessels was present in rest of the 12 patients (23%) (Figure 4, 5). None of the patients had shown culprit artery severe disease or clot burden on angiograms. All the patients were managed medically and ECG changes started resolving in most of the patients with Ischemia Guided Medical Therapy while being hospitalized. Follow up was advised after two weeks for any worsening anginal symptoms or persistent ECG changes (Table 2).

Table 2: Signs, Symptoms and Demographics

Variables	Our Study
Demographics	
Age distribution	30-60 years (87% in b/w 30-45yrs
Gender distribution	Males: 62%, Females: 38%
Signs and Symptoms	
Chest pain with or without dyspnea	38%
Risk Factor Profile	
Previous episode of angina or MI	None
Diabetes	59%
Hypertension	12%
Investigations	
Rise in acute Inflammatory markers	82%
Quantitative Troponin I levels in high probability range	78%
ECG: ST-Segment elevations	87% (Anterior leads: 67%)
Ejection fraction <45%	96%
Segmental motion wall abnormality	0%
Angiography	Culprit related artery was normal or re-canalized
Patients who underwent Thrombolysis	80%
Investigations	
Patient outcome	Good prognosis & low mortality (Owing to low risk factor profile, less critical Pneumonia, early resolution of respiratory findings and early mobility of patients)

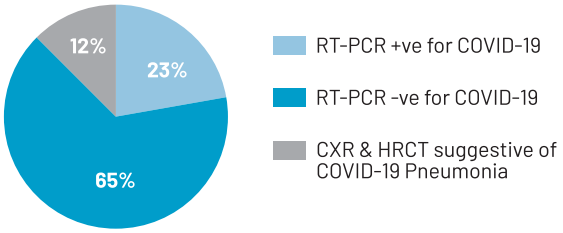

Figure 1: COVID-19 status at the time of Angiography

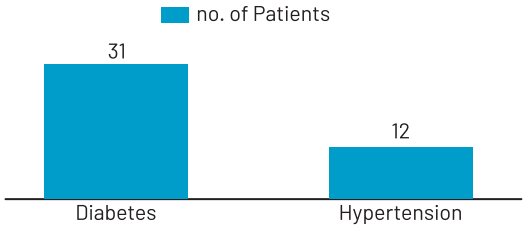

Figure 2: Frequency of co-morbidity

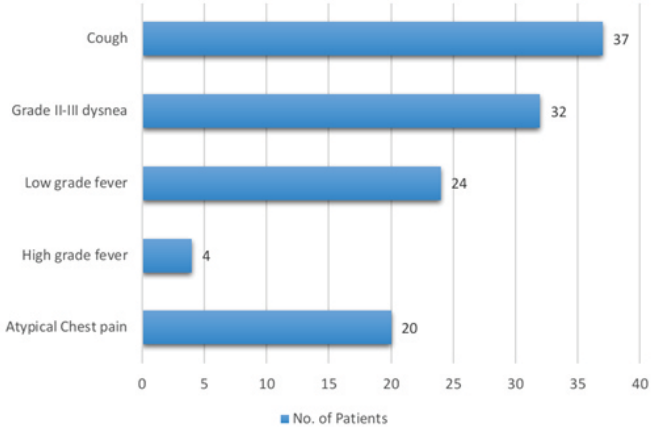

Figure 3: Clinical presentation of patients

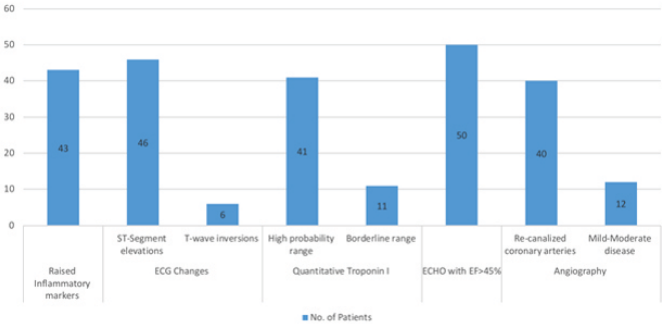

Figure 4: Clinical investigations of the patients

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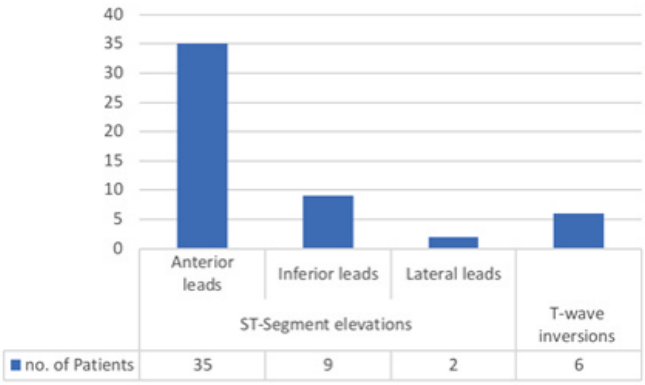

Figure 5: Electrocardiogram Changes

DISCUSSION

This is a first case series study conducted locally to assess related thrombotic cardiovascular events in COVID-19 patients. A lot of cases have been reported internationally showing probable hypercoagulable state in these patients and mimicking ST-elevation myocardial infarction. All the cases under study were discharged on medical management of anti-ischemic therapy and anticoagulation for short time. All the patients remained stable and no mortality was recorded due to cardiovascular cause. As evidenced by different case reports by Mustafa *et al.*, and Mengal *et al.*, our patients were also in the age group of 30-60year, but the striking factor was that most of the patients (87%) were in the 30-45 years of age group [9, 10]. The distribution of gender was same as various studies in literature review show, having a greater proportion of males (62%) than females (38%). Stefanini *et al.*, have discussed that most of the patients (78%) had a complaint of chest pain with or without dyspnea whereas, our patients reported chest pain in only 38%. However, 46% of the patients reported low grade fever with a median time of 5-10 days before the other symptoms or ECG changes [11]. We observed the raised D-Dimers and CRP level in these cardiac patients in about 82% cases as evidenced by studies conducted by Karbalai *et al.*, in China, and Tahir *et al.*, which also show a comparative rate of rise of acute inflammatory markers in 90% of the cases [12, 13]. All the patients were tested for COVID RT-PCR before proceeding in line with the guidelines of COVID-19 diagnosis supported by study on the epidemiological and clinical features of Corona virus disease conducted by and Xu *et al.*, and Wang *et al.*, [6, 7]. Out of these cases, about 12 patients (23%) were known positive before proceeding for angiography and underwent procedure after stabilization. Almost 34 patients (65%) were positive on PCR about 2 weeks before and were negative on PCR at the time of the coronary angiography. Whereas the rest of 06 patients were under high suspicion for their typical CXR and HRCT findings for COVID-19 pneumonia and were proceeded for angiography after initial stabilization for pneumonia. None of the patients had previous episode of angina or myocardial infarction but 59% of the patients had diabetes in their comorbid risk factors and only 12 patients had hypertension which is consistent with the results of the studies by Xu *et al.*, Wang *et al.*, He *et al.*, and Xiong *et al.*, [6, 7, 14, 15]. Total 87% of the patients had findings of ST-segment elevation in either anterior, inferior or lateral leads with more (67%) being in anterior leads, which is further supported by the studies and case report by Inciardi *et al.*, and Stefaninin *et al.*, Haussner *et al.*, where is the ration is about 70% for the anterior lead changes [4, 11, 16]. However, T wave inversions were only found in 06 of our patients. As per criteria of diagnosis of Myocardial infarction and AHA guidelines, all patient with ECG changes were tested for quantitative Troponin levels and levels were raised for all of them. Our 78% patients had high probability values as compared to 92% rates in other studies by Vetta *et al.*, Chen C *et al.*, and Bujak *et al.* [2, 5, 8]. The Echocardiographic findings of normal segmental wall motion analysis and ejection fraction greater than 45% reported in our study is in contrast to other studies available by Inciardi *et al.*, Bujak *et al.*, Stefaninin *et al.*, Tahir *et al.*, Bangalore *et al.*, which reported about 25-35% patients with some motion wall abnormality and 14-20% patients with ejection fraction less than 45 [4, 8, 11, 13, 17]. A striking finding of this study is all of the patients recruited underwent coronary angiography and the culprit related artery was normal or recanalized with no obstructive lesion, which is strongly supported by all the studies and case reports by Bujak *et al.*, Tahir *et al.*, and Stefanini *et al.*, Haussner *et al.*, Banagalore *et al.*, in which COVID-19 virus is reported to be causing ST elevation myocardial infarction [8, 13, 11, 16, 17]. However, none of the patients in these studies underwent thrombolysis but our 42 patients underwent thrombolysis with streptokinase in Emergency department. In accordance with the studies mentioned, all the patients were kept on Heparin infusion in the pre-procedure phase and direct Xa inhibitor anticoagulants were given for 6 weeks post procedure along with single antiplatelet agent as outlined in the guidelines erected in the study by Bikdeli *et al.* [18]. The good prognosis and low mortality rate in our patients can be supported by low risk factor profile and less critical pneumonia in term of intermittent low flow oxygen inhalation need, early resolution of respiratory findings and early mobility of the patients as compared to poor outcomes reported in cardiovascular complications by Shi *et al.*, Yang *et al.*, and Li *et al.*, [3, 19, 20].

CONCLUSIONS

We can conclude that acute Cardiac injury in previously asymptomatic patients may be related to the pro-thrombotic and hypercoagulable state created in the body

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by COVID-19 infection which leads to endothelial dysfunction and mimics ST-elevation myocardial injury raising Troponin levels. The raised D-dimer and CRP levels indicate that oxidative stress, cytokines and inflammatory interleukins might be involved in the pathophysiology. The auto-recanalization of the coronary arteries and normal echocardiographic findings, are good prognostic factors for the post cardiac injury rehabilitation, which can further be enhanced by timely management and careful monitoring along with guideline directed therapy.

Authors Contribution

Conceptualization: AT
Methodology: AJ, AF
Formal analysis: ZZ, SN. YK
Writing-review and editing: AT, AJ, AF, ZZ, SN, YK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Determining Genetic Variability and Taxonomy of *Hibiscus Rosa-sinensis* Through Rbcl Molecular Marker

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ABSTRACT

Medicinal plants have been used in traditional medicine for a long time. These plants contain phytochemicals that have a variety of medicinal properties. However, accurate identification and authentication of medicinal plant species ensured their safety and efficacy. DNA barcoding using molecular markers has proven to be a useful method for plant species identification. The rbcL molecular marker was used for detailed characterization, amplification, and phylogenetic studies of *Hibiscus rosa-sinensis*. **Objective:** To evaluate the therapeutic properties and potential applications of *Hibiscus rosa-sinensis*. **Methods:** Samples of *H. rosa-sinensis* were collected, and DNA was isolated by the Doyle and Doyle method. The presence of DNA was confirmed by gel electrophoresis, and specific primers were used for PCR amplification. The PCR results were sequenced using next-generation sequencing techniques. After that, a neighbor-joining technique was used for phylogenetic analysis and to obtain pairwise nucleotide distances. Gel electrophoresis confirmed the presence of DNA in plant samples, and PCR amplification using rbcL primers generates successful amplification results. **Results:** The obtained sequence was 99.7% identical to the previously reported rbcL gene sequence from *H. rosa-sinensis*. Based on phylogenetic research, *H. rosa-sinensis* was discovered as a closely related species. **Conclusions:** The rbcL gene has been found as a viable molecular marker for *H. rosa-sinensis* identification and phylogenetic analysis. The results of this study demonstrated the therapeutic potential of *H. rosa-sinensis* and the importance of species identification in herbal medicine. DNA barcoding proved a reliable authentication and quality control technology in the herbal medicine business.

INTRODUCTION

Medicinal plants, known as medicinal herbs, have been used in traditional medicine since ancient times. These plants produce a variety of chemical compounds for various purposes, such as protection against insects, fungi, diseases, and herbivorous mammals [1]. Various potentially effective phytochemicals have been discovered. However, due to the wide range of phytochemicals in a single plant, using whole plants for medicinal purposes requires further investigation. Furthermore, thorough scientific research is required to evaluate the phytochemical content and pharmacological properties of many plants with medicinal potential to determine their efficacy and safety [2]. Medicinal plants are widely used in modern and traditional medicine to maintain health and treat specific conditions. According to Food and Agriculture Organization estimates, in 2002, the global use of medicinal plants exceeded 50,000 [3]. The use of medicinal plants has threefold benefits. First, they provide health benefits to those who use them medicinally. Second, they offer financial benefits to individuals who harvest, process and distribute these plants commercially. Finally, society benefits from medicinal plants through job creation, tax revenue and a healthy workforce [4]. The Malvaceae family is found in both tropical and temperate regions. About 125 Malvaceae species have been reported in Asia, belonging to 22 genera [5]. *Hibiscus rosa-sinensis*, commonly called China rose and belonging to the Malvaceae family, is a plant of crucial medicinal value. It is

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an effective remedy for wounds, inflammation, fever, cough, diabetes, bacterial and fungal infections, hair loss, and stomach ulcers in tropical regions [6]. Studies have shown that *Hibiscus* flowers have anti-tumor properties and can be used as analgesic, antipyretic, anti-asthmatic, and anti-inflammatory agents. Additionally, these flowers are rich in antioxidants and exhibit potent antimicrobial and antifungal activity [7]. Studies on various parts of *Hibiscus*, including extracts of its stems, roots, leaves and flowers, have revealed the health benefits of its phytochemical constituents. These ingredients exhibit antioxidant activity, effectively scavenging free radicals in the body that can potentially damage DNA [8]. Figure 1 shows the flower and plant perspective of *H. rosa-sinensis*, which is highly valued in the economy for its herbal and medicinal uses[9]

Figure 1: (a) Plant view (b) Flower view

Table 1: Morphological characteristics of *Hibiscus rosa-sinensis L.*

Common names	China rose, Shoe flower, Gudhal, Jasum, Gul gurhal taza [10]
Family	Malvaceae
Habitat	The world's tropics and sub-tropics regions range from 30 degrees north latitude to 30 degrees south latitude.
Distribution in Pakistan	It is extensively cultivated as an ornamental plant in tropical areas of Pakistan.
Worldwide Distribution	China, India, Nepal, Africa
Habit	Shrubs
Life form	Perennial
Flowering Period	Throughout the year
Morphological characters	The plant can grow to a height of 1-5m and has a woody stem with a globe shape. The **stem** has branches and is sparsely pubescent with simple dark hairs. The plant **leaves** are broadly lanceolate, serrate, lobed or ovate, with linear stipules 5-10 mm long and lanceolate. The **petiole** measures 0.5-2 cm. The plant's flower is axillary and solitary, with an erect or sub-pendulous side. The single or double pedicel is 1-8 cm long and conspicuous near the top. The flower centre can be yellow to purple, and the peduncle is 1-4 cm long, with a firm and dense fruit. The **calyx** of the plant is 5-lobed, tubular-campanulate, and 1.5-2 cm long. The shape can range from triangular to lanceolate. The **corolla** is 5-lobed and measures 4-9 cm. It can appear pink to red, with or without dark central petals. Petals are ovate or oblong, 5-9 cm long and 3-7 cm wide. The **stamen** is short or almost as long as the petals and has a broad base. The apex of the filaments is either truncate or 5-dentate, and the anthers are basally fixed, entire or at the apex. The **carpel** of the plant is superior, 5-locular to rarely 10-locular. It has axillary placentation with ovules that are 3 or more per locule. The **style** measures 1 or 5 branches apart, with a discoid-capitate or indehiscent stigma. The fruit of the plant is a capsule of globose-cylindrical shape with an apex that is apiculate, acute or acuminate. It fades locally and may be glabrous or hairy. The plant produces many reniform or subglobose seeds that may be smooth or hairy [11].
Ethnobotanical uses	This herb exhibits astringent properties and has a cooling effect. It has the ability to control bleeding, soothe irritated tissues and relax muscle spasms. The herb's flowers have astringent, aphrodisiac, and refrigerant properties. They are used internally to treat diseases such as cystitis, bronchial catarrh, excessive and painful menstruation, febrile illness, Intestinal diseases and cough. The flowers are also used for hair growth and as a cooling drink for sick people. Additionally, they work to treat mumps, fever, and wounds. The herb leaves have anodic, emollient, and laxative properties and are used externally as a lotion in treating fever [10].

The unscrupulous practice of compounding herbal medicines has eroded trust in recent decades. It involves mixing or replacing a specific medicinal plant with a morphologically similar plant that lacks therapeutic properties. Such unethical practices have led to declining the efficacy of herbal remedies and, consequently, losing faith in treatment methods [12]. Adulteration is a common problem in the herbal medicine industry that poses significant health risks to consumers and severely affects the industry. Medicinal plants are commonly purchased in markets and traditional herbal shops in various forms, such as dried leaves, roots and bark, and processed mixtures or extracts. To ensure accurate identification by retailers and customers, plant material must have appropriate morphological characteristics [13]. In the current literature, the term 'DNA barcoding' has emerged as a new name for a concept that has been used for a long time [14]. A quick and accurate species identification method involves using a standard DNA segment as a marker [15]. DNA barcoding is a globally recognised technique for species identification, which involves using specific regions of DNA and established protocols to generate a comprehensive database of organisms [16]. The importance of plant DNA barcoding is rooted in the need for accurate species identification, which is vital for plant conservation and utilisation. However, this process may require extensive taxonomic knowledge in multiple regions worldwide [17]. DNA barcoding can provide an accurate and reliable alternative to morphological identification for biological material and is often used when identification by macroscopic or microscopic methods is difficult [18]. This technology can differentiate and classify species at any stage of their development or processing as long as DNA extraction is possible [19]. DNA barcoding in plants is currently being applied in many applications. Different methods have been used to authenticate plant products, such as medicinal plants [18], kitchen spices [20], berries

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[21], olive oil [22], and tea [23]. Phylogenetic trees for community ecology are constructed using genetic sequences obtained by DNA barcoding [24]. Identification of different species depends on the use of specific regions of DNA. It is crucial to accurately document the laboratory procedures used during sample processing along with the primers used. End users can access DNA sequencing trace files and quality statistics [25]. GenBank and the Barcode of Life Data System (BOLD) are viable options for collecting DNA sequences because all data are publicly available. However, it is recommended to use bold for this purpose [17]. BOLD offers a comprehensive solution for project management by enabling the storage of DNA sequences and trace files [17]. The present study aims to create awareness about the importance of using natural medicinal plants, the prevalence of adulteration and the impact of the quality of certain medicinal plants on human health. Promoting the treatment and export of herbal plants can improve the economy and maintain the quality of a particular herbal medicine. This study provides resources for information on techniques used to detect the presence of adulterants in plants, such as DNA barcoding.

METHODS

Hibiscus rosa-sinensis plant was obtained from Minchin Abad Tehsil of Bahawalnagar district located at 30°05'53.1"N 73°37'28.4" E. Taxonomic expert Prof. Dr Muhammad Zia-ur-Rehman carried out the initial identification procedure using the available flora [26]. Collected plants were stored in zip lock bags carefully labelled with the location and date of collection, common name and identifier. The plants were then transported to the Plant Molecular Lab of the Department of Botany, Government Graduate College of Science, Lahore, where the leaves were stored in a -20°C freezer for further processing. DNA analysis was performed to detect any possible adulteration in the plant sample, which is the most accurate and sensitive method. Plant samples were processed for DNA analysis, and a protocol for admixture detection was developed based on the results. The Doyle and Doyle method was used for DNA extraction [27]. A sterile ice-cold pestle and mortar were used to crush 1 g of sample with liquid nitrogen to extract genomic DNA from plant samples. The resulting material was transferred to an Eppendorf, and 750 µl of Cetyltrimethylammonium Bromide (CTAB) was added. The mixture was then incubated in a water bath at 65 °C for 30–45 min. Then, a solution of equal volume of chloroform and isoamyl alcohol (24:1) was added to Eppendorf and centrifuged at 10,000 rpm for 15 min. The supernatant was then transferred to a new Eppendorf tube, and 750 µl of chloroform and isoamyl alcohol solution was added. The mixture was then centrifuged at 10,000 rpm for 15 min. This process was repeated three times, and the clear supernatant was collected in another Eppendorf. 2/3 volume of ice-cold isopropanol was added to each Eppendorf-containing supernatant to precipitate the DNA strands. The DNA strands were visualised as minute threads and left to Eppendorf overnight in a freezer at -20°C. The following day, the Eppendorf tubes were centrifugated at 10,000 rpm to allow the DNA strand to settle to the bottom as a pellet. The supernatant was discarded, ensuring the safety of the pellet. The pellet was washed three times with 200 µl washing buffer and then allowed to dry. Next, the DNA was dissolved in 50–70 µl of distilled water. Two methods were used to confirm the presence of plant DNA samples. DNA samples' concentration, purity and quantity were confirmed by measuring the absorbance at 260 nm using a nanodrop spectrophotometer (Thermo Scientific, Wilmington, USA). Finally, DNA samples were typically diluted 20 times (dilution factor) but varied based on DNA concentration. A Halo DB20 double-beam spectrophotometer model was used for DNA quantification, with TE buffer as the reference buffer. Additionally, optimizations were made according to the respective protocols [28]. Gel electrophoresis is a valuable technique for the identification of plant DNA. A 1% agarose gel was formed using 0.25 g agarose powder and 25 ml of 1X TAE buffer. The mixture was heated in a microwave oven for one minute until it became transparent and then cooled to 60 °C. Ethidium bromide was added to the liquid gel at a 0.5 µg/mL concentration. The liquid gel was then poured into a tray with two appropriately sized combs and allowed to solidify. The gel tray was then immersed in a gel electrophoresis tank containing 1X TAE buffer. The combs were carefully removed, and 10 µl DNA sample and 4 µl loading dye were mixed and loaded onto the gel with a 100 bp ladder (Thermo Fisher Scientific, USA). The gel was then run at 100 volts for 30 min and observed under UV illumination in a gel documentation system (Bio-Rad, USA), which captured images. The bands in the gel indicate the presence of DNA in the samples [29]. After DNA confirmation, PCR was used to amplify all DNA samples. Extracted genomic DNA extracts were prepared using the equation $M_1V_1=M_2V_2$ and double-distilled water. Highly efficient PCR techniques were used to amplify specific gene regions of plant DNA, including matK, rbcL, nrITS, and trnH-psbA, using a master mix and appropriately selected primers. PCR of the plant DNA sample was performed with appropriate primers [30]. Table 2 lists the primer sequences that were selected based on a thorough review of the literature.

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Table 2: Short Oligonucleotide Primers

Barcode	Primer	Primer sequence	Reference
matK	matK F	5′-TAATTTACGATCAATTCATTC-3′	[31]
	matK R	5′-CTTCCTCTGTAAAGAATTC-3′	
rbcL	rbcL F	5′-ATGTCACCACAAACAGAAAC-3′	[32]
	rbcL R	5′-TCG CAT GTA CCY GCA GTT GC-3′	
nrITS	nrITS F	5′-CCTTATCATTTAGAGGAAGGAG-3′	[33]
	nrITS R	5′-GGAAGTAAAAGTCGTAACAAG-3′	
trnH-psbA	trnH-psbA F	5′-GTTATGCATGAACGTAATGCTC-3′	[34]
	trnH-psbA R	5′-CGCGCATGGTGGATTCACAAATC-3′	

25 µl of the reaction mixture was aliquoted in PCR tubes. The PCR profile was set with an initial temperature of 94 °C for 3 min, an annealing temperature of 48 °C for 1 min, and an extension temperature of 72 °C for 10 min. All temperature settings were maintained to run for 40 cycles. The PCR product (5 µl) was mixed with 3 µl loading dye (5X) and run on a 1% agarose gel to confirm DNA amplification (Figure 2).

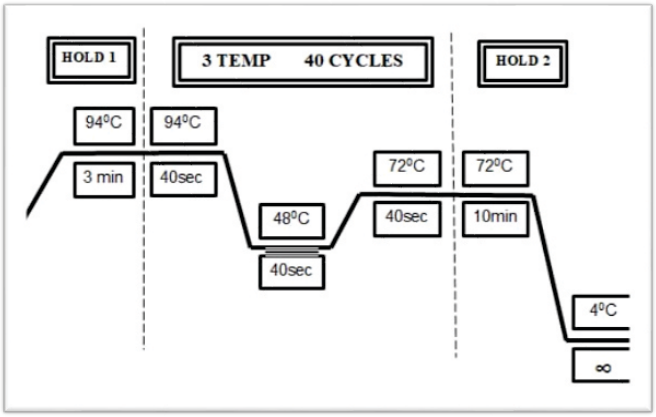

Figure 2: Steps of PCR with conditions

After DNA amplification, the next step involved purifying the plant DNA sample. To achieve this purity, distilled water was taken into the Eppendorf containing the DNA, resulting in a total volume of 100 µl. Equal volumes (100 µl) of the phenol and chloroform mixture were added to the same Eppendorf, and the entire mixture was inverted. Eppendorf tubes were centrifuged at 14000 rpm for 6 min to obtain the top aqueous phase of the solution, which was then transferred to a new tube. To further purify the DNA, 9 µl of a 3 molar sodium acetate solution (1/10th volume, pH 5.2) and 250 µl of absolute ethanol (2.5% volume) were added to Eppendorf and stored at -20 °C. But was left for 30 minutes. The tubes were centrifuged at 14000 rpm for 10 minutes to precipitate the DNA and extract ethanol. The DNA pellet was washed with 100 µl of 70% ethanol, centrifuged at 14000 rpm for 2 min, and the ethanol was removed. After isolation of the DNA pellet, it was essential to keep it dry and dissolve it in 20 µl of sterile distilled water (SDW). This method has proven to be highly effective in purifying plant DNA samples. To confirm DNA purification, 4 µl of DNA solution was loaded onto a 1% agarose gel. Purified PCR products were then sent to "CELEMICS BTSeq™, Seoul, Korea" for sequencing. The results were analyzed through a comparative study. Next-generation sequencing (NGS) was used instead of Sanger sequencing because it is more accurate and reliable. NGS is a massively parallel sequencing technique that offers extremely high throughput, scalability, and speed. Therefore, this technology was used to determine the sequence of nucleotides in the entire genome or targeted regions of DNA or RNA [35]. To identify closely related sequences, we performed a blast search on NCBI. For DNA analysis, we downloaded twelve adjacent sequences from plant samples. To perform phylogenetic analysis, we used the neighbor-joining method [36] with MEGAX software [37]. As part of the phylogenetic tree construction process, a bootstrap test was run with 1000 replicates.

RESULTS

DNA samples were analyzed by agarose gel electrophoresis, allowing the gel to run at 100 volts for 30 min. A gel documentation system (Bio-Rad, USA) was used to observe the gel under UV illumination, and the presence of bands in the gel confirmed DNA in the samples (Figure 3).

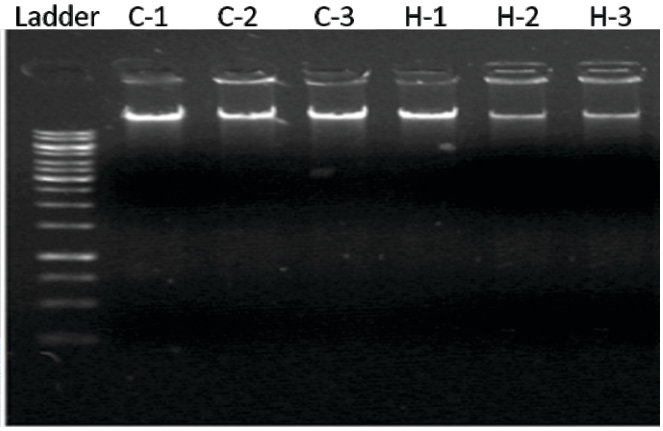

Figure 3: 1% Gel electrophoresis showing DNA profile of Plant samples

The DNA concentration of the plant samples was measured using a spectrophotometer at a wavelength of 260 nm, with distilled water as a reference. The concentration was calculated using the formula provided [28]. DNA concentration (µg/ml) = E × OD260 × dilution factor. (E is extinction coefficient = 50 for dsDNA) (Table 3).

Table 3: Concentration of DNA at 260 nm

Serial. No	Sample ID	OD at 260 nm	DNA in µg/mL
1	C-1	0.7276	1491.58
2	C-2	0.658	1348.9
3	C-3	0.655	1342.75
4	H-1	0.587	1203.35
5	H-2	0.606	1242.3
6	H-3	0.62	1271

After analysing the OD values at 260 and 280 nm wavelength using the formula Quality of DNA = OD_{260}/OD_{280},

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we can confirm the quality of the DNA. Usually the net ratio of DNA is about 1.8 [38]. However, if the ratio falls below 1.6, it may indicate the presence of contaminants, such as proteins or phenols, which absorb at or near 280 nm. Based on this, C-1 and H-1 were selected for further work [39], as shown in Table 4.

Table 4: Quality of DNA at OD 260/280

Sample	OD at 260	OD at 280	Ratio
C-1	0.7276	0.404	1.80
C-2	0.658	0.369	1.78
C-3	0.655	0.370	1.77
H-1	0.587	0.326	1.80
H-2	0.606	0.344	1.76
H-3	0.62	0.349	1.79

DNA barcoding primer pairs (matK, rbcL, nrITS, and trnH-psbA) were used to PCR the plant DNA sample on a Bio-Rad thermocycler [30]. In a 1% agarose gel electrophoresis procedure, 10 µl of the PCR product was used, and a band of the desired size was successfully obtained. Notably, it was observed that only the rbcL primer pair yielded positive results for the plant sample, as shown in Figure 4.

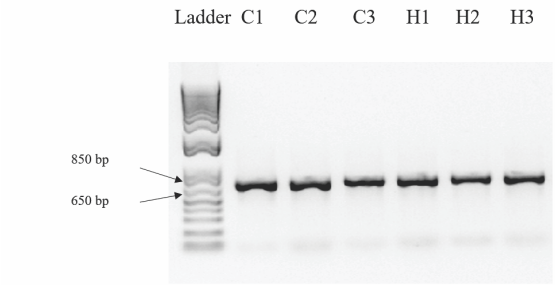

Figure 4: 1% Agarose Gel electrophoresis of PCR product representing the DNA bands of *Hibiscus rosa-sinensis*

The DNA sequences from the rbcL primer were edited and assembled through EditSeq software from DNA Star. Afterwards, these sequences were utilised to perform a blast on the NCBI database (https://www.ncbi.nlm.nih.gov/) to download related sequences. The evolutionary history of the sequenced sample was determined using the neighbor-joining method [36]. The muscle method in MEGA11 software was used to align multiple sequences for homology analysis. Pairwise nucleotide identities were calculated for phylogenetic analysis using MEGALIGN (DNA Star) software. The rbcL gene sequence from *Hibiscus rosa-sinensis* was found to have 99.7% pairwise nucleotide sequence identity with the previously reported rbcL gene sequence from *Hibiscus rosa-sinensis* [NC_042239], *Hibiscus rosa-sinensis* [MK382984], and 98.8 % *Hibiscus mutabilis* [MK820657] that was reported from Islamabad. Figure 5 illustrates these results.

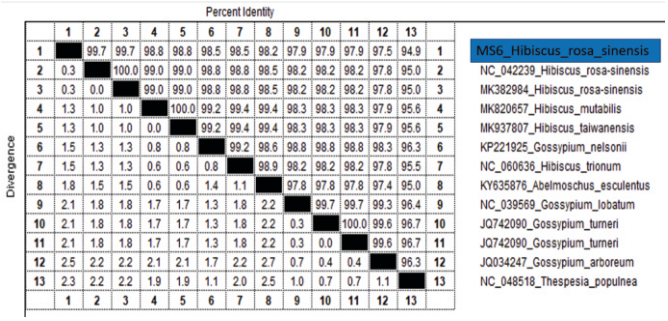

Figure 5: Sequence distancing of *Hibiscus rosa-sinensis*

DISCUSSION

Specimens of *H. rosa-sinensis* were systematically collected from different Punjab, Pakistan regions and were appropriately labelled with name, locality, and collection date. The samples were then stored in zip lock bags and sent in an ice box to the Molecular Biology Laboratory of the Government Graduate College of Science at Wahdat Road Lahore for morphological characteristics, including stems, leaves, flowers, seeds, and families. A standard protocol was used for DNA extraction, and confirmation was performed by 1% gel electrophoresis. The extracted DNA was further amplified by polymerase chain reaction (PCR) using primers including matK, rbcL, psbA-trnH, and ITS. In 2009, CBOL (The Consortium for the Barcode of Life) proposed that rbcL and matK be used as the standard plant DNA barcode consisting of two loci. Moreover, the psbA-trnH spacer and the nuclear internal transcribed spacer 2 (ITS2) are primarily functional [21, 40, 41]. The amplification of selected plants using the rbcL primer (750 bp) was successful, and the obtained amplifications were used for further processing. PCR products were purified, and nucleotides from the rbcL gene were sent to "CELEMICS BTSeq™, Seoul, Korea" for sequencing. Nucleotide sequencing was performed by next-generation sequencing. The rbcL gene has been identified as an efficient DNA barcoding tool for plants of the Malvaceae family, including *H. rosa-sinensis* [39]. The molecular marker rbcL gene has effectively identified processed plant products used in various fields such as food, medicine and cosmetics [42]. This information can significantly benefit experts who rely on accurately identifying plant products. The results agree with previously documented rbcL gene sequences, indicating a high level of consistency [39, 42]. Through an NCBI BLAST search on each sample sequence in GenBank, it was found that the closest matches to the same species were at 99% similarity. The rbcL gene sequence obtained from *Hibiscus rosa-sinensis* showed high similarity with previously reported sequences of the same species, with a percentage nucleotide sequence identity of 99.7%. Furthermore, it showed 98.8% similarity with the rbcL gene of *Hibiscus mutabilis*. The

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studied plants, particularly *H. rosa-sinensis*, were confirmed by NCBI BLAST and by examining different plants for their morphology. In addition, cross-matching was done by investigating different subjects. We analyzed pairwise nucleotide distances between selected plant species within and between species. The results showed that the homology percentage of nucleotides between different species was higher than within the same species. This allowed us to infer that a species exhibits high homogeneity with its closely related species. To develop a successful DNA barcode, it is essential to distinguish between intraspecific and intraspecific divergence [43]. Mitochondrial COI in animals shows more significant variation within species than between species, resulting in a 'barcode gap' that allows reliable species differentiation. However, the plastid DNA of land plant regions mark and rbcL do not provide the same 'barcode gap', especially in closely related species [44, 40]. A recent study successfully extracted DNA from seven infected samples and confirmed their presence by gel electrophoresis. Notably, three of these samples exhibited amplification by PCR reactions, specifically containing a beta-satellite component at 1.4 Kbps (S3, S4, and S6). Each of the three amplified samples was subjected to restriction enzyme assays with Bam H1, ECOR1, and Pst1, yielding different bp length results [45]. During our research, we analyzed intraspecific and interspecific sequence divergence. We observed a significant overlap of sequences, which did not require further analysis to identify a barcode gap. Although the barcode gap and distance method have been used to differentiate plant groups beyond the species or genus level, it is well documented that matK and rbcL barcodes differ among plants at the species level however, specific barcodes don't show the difference [46]. Cluster analysis was performed, and NJ trees were constructed with bootstrap analysis to assess the effectiveness of barcodes in distinguishing between species [47, 48]. The DNA sequence of the rbcL gene was used to construct a phylogenetic tree, which showed that *Hibiscus rosa-sinensis* (MS06) shared a clade with *Hibiscus rosa-sinensis* and *Hibiscus mutabilis,* whose bootstrap values are 99.7% and 98.8%. Our study demonstrated that the rbcL gene can correctly identify *H. rosa-sinensis*. The high discrimination efficiency of the gene for this species makes it a valuable barcode for identification purposes. Its short sequence length makes it an informative and powerful molecular tag for distinguishing weed and cultivated plant species.

CONCLUSIONS

The use of herbal products is widespread in various spheres of life. However, it is imperative to exercise caution and confirm their identity, as they can adversely affect human health. It is important to ensure that the herbal products are authentic and not fake, which can be used to fulfil personal goals. Molecular identification, especially DNA barcoding, can be a valuable technique to distinguish between plant species or plant-derived products, thus ensuring their efficient use in diverse fields.

Authors Contribution

Conceptualization: AI
Methodology: MZUR
Formal analysis: AI
Writing-review and editing: AI, MZUR

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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