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Comparison of the Frequency of Raised Level of Anti-Thyroid Peroxidase Antibody in the Patients of Hypothyroidism and the Euthyroids

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ABSTRACT

The goal of this study was to compare the frequency of raised level of anti-thyroid peroxidase antibody in patients of hypothyroidism and the euthyroids. This case-control study was carried out at the department of Pathology, Quaid e Azam Medical College/Bahawal Victoria Hospital, Bahawalpur, from October 1, 2018 to Setember 30, 2019 and the subjects were selected by the non-probabilty consecutive sampling technique. The frequency of raised level of anti-thyroid peroxidase antibody in patients of hypothyroidism and the euthyroids was compared. In the present study, the mean age of the patients with cases of hypothyroidism was 32 ± 10 years, and the mean age of controls was 32 ± 10 years. Raised anti-thyroid peroxidase antibodies were found in 20 (28.99%) cases and 5 (7.25%) controls. After applying a Chi-squared test, a statistically significant (P = .00) difference in the level of anti-thyroid peroxidase antibodies between the cases and controls was detected. The presence of raised anti-thyroid peroxidase antibodies was also significantly associated with age and female gender. In conclusion the early screening of the anti-thyroid peroxidase antibodies specially in women above 30-years would notably affect the outcome of the disease with congruent disease management.

Keywords: Hypothyroidism, euthyroids, anti-thyroid peroxidase antibody

INTRODUCTION

Hypothyroidism is characterized by abnormally low serum thyroxine (T3) and tri-iodothyronine (T4) levels and a high thyroid-stimulating hormone (TSH) level. The defect that makes a person likely to develop an autoimmune thyroid disease is still unknown. Some suggest a precise tissue defect in the suppressor T-cell function, an idiotype/anti-idiotype reaction, and a biologically programmed antigen (Friedrich et al., 2008). The existence of Anti-thyroid peroxidase (anti-TPO) antibodies and concurrent autoimmune hypothyroidism is well-documented (Zelaya et al., 2010). According to a survey by the Amrita Institute, Cochin, 9.5% of subjects (of 19.6% of individuals suffering from thyroid function abnormalities) had anti-TPO antibodies (Unnikrishnan et al., 2011). The reported prevalence of subclinical hypothyroidism in men and women is 3% and 8%, respectively. The risk of the development of clinical hypothyroidism is 4% annually if sub-clinical hypothyroidism is related to positive anti-TPO antibodies (Jameson et al., 2018). A research conducted in Muzaffarabad indicated no age-related link between anti-TPO antibodies level, but found association with high levels of TSH (Naz et al., 2009).

In diagnosis and effective treatment of autoimmune thyroid diseases, the assessment of the presence of anti-TPO

antibodies is important. According to a research in 2007, up to 74.5% and 56% of cultured thyroid cells can be harmed by antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity of anti-TPO antibodies, respectively (Larsen et al., 2003). Monocytes, via their Fc-gamma receptor 1 (FcγRI), are important effector cells in ADCC mediated by anti-TPO antibodies and may, with T cells, contribute to the destruction of the thyroid gland in autoimmune thyroid disease (Rebuffat et al., 2008). The clinical diagnosis of autoimmune thyroid disease is usually confirmed by the detection of various antibodies in the patient's blood samples (Chen et al., 2011). Elmugadam AA et al in their study showed that anti-TPO antibody of thyroid disease patients and the control group was positive in 21.2% and 5% respectively (Elmugadam et al., 2010).

The existence of anti-TPO antibodies may indicate future thyroid diseases. This study was planned to see the raised anti-TPO antibody in cases of hypothyroidism and in the euthyroids so that some practical recommendations can be made for preemptive prevention of developing hypothyroidism through an early detection of the anti-TPO antibody with subsequent early management by immunosuppressive drugs.

MATERIALS AND METHODS

This case-control study was carried out at the Department of Pathology, Quaid e Azam Medical College/Bahawal Victoria Hospital Bahawalpur from September 1, 2018, to August 31, 2019. Patients with hypothyroidism were diagnosed by measuring for free T3 (FT3) and free T4 (FT4) below the lower limit of the normal range (normal range of FT3: 3.2 to 8.0 pmol/L; normal range of FT4: 10.3 to 34.7 pmol/L) and TSH above the upper limit of the normal range (normal range of TSH: 0.4 to 4.2 mIU/L) served as cases for the study. The subjects were selected through non-probability consecutive sampling technique.

Individuals that served as controls were euthyroid and labeled as such when the person had no signs and symptoms for the disease and FT3, FT4, and TSH were within the normal range (normal range of FT3: 3.2 to 8.0 pmol/L, normal range of FT4: 10.3 to 34.7 pmol/L and normal range of TSH: 0.4 to 4.2 mIU/L).

Anti-thyroid peroxidase autoantibodies targeted against the thyroid peroxidase (TPO) enzyme having serum cut off values ≥10 IU/ml were labeled as raised anti-thyroid peroxidase antibodies and < 10 IU/ml was taken to be normal.

A total of 69 hypothyroid patients aged 18 to 60 years of both genders were selected. Age and sex-matched euthyroids served as controls. Any patients taking medicine for thyroid disease, patients with hyperthyroidism, thyroiditis or thyroid malignancy, having any other medical illnesses such as renal diseases, cardiac diseases, hepatic diseases, pulmonary diseases, or any neoplastic disease, patients having an autoimmune disease or on immunosuppressants, and pregnant females met the exclusion criteria of our study.

This study was approved by the hospital ethical committee, and written informed consent was provided by all patients. Patients were recruited from the medical outpatient department of Bahawal Victoria Hospital, Bahawalpur, Pakistan. Both cases and control groups had 5 ml of blood drawn, which was immediately transferred to red-top or gel tubes. Samples were allowed to clot for one hour prior to centrifugation. Test serum was clear and non-hemolyzed. Serum was analyzed via a fully automated analyzer using enhanced chemiluminescence method to detect serum FT3, FT4, and TSH levels. Anti-thyroid peroxidase antibodies were measured by

enzyme-linked immunosorbent assay. A standardized form was used to collect pertinent information from every patient.

Data were collected and analyzed using IBM SPSS Statistics version 20.0 (Armonk, NY: IBM Corp). Numerical data were presented as mean and standard deviation (SD). Categorical data were presented as frequencies and percentages. A Chi-square test was applied to detect a difference in raised anti-thyroid peroxidase antibodies between the cases and controls. P value ≤ .05 was taken as significant.

RESULTS

In this study, the mean age of the cases was 32 ± 10 years, and the mean age of controls was 32 ± 10 years. Raised levels of anti-thyroid peroxidase antibodies were found in 20 (28.99%) cases and 5 (7.25%) controls. After applying a Chi-squared test, a statistically significant (P = .00) difference in the level of anti-thyroid peroxidase antibodies between the cases and controls was detected (Table 1)

Table 1: Comparison of raised anti-thyroid peroxidase antibodies in cases and controls

Group	Raised Anti-thyroid peroxidase antibodies		Total	*P*-value
	Yes (%)	No (%)		
Cases (Hypothyroids)	20 (28.99)	49 (71.01)	69	.00
Controls (Euthyroids)	5 (7.25)	64 (92.75)	69	

The evaluation of age distribution of the selected patients was done, and two groups were formed: an 18 to 30-year-old age group and a 31 to 60-year-old age group. In the 18 to 30 group, a total of 37 (53.62%) were cases and 41 (59.42%) were controls. Raised anti-TPO antibodies were noted in 9 (24.32%) cases and in 2 (4.88%) controls in the said age group. The difference in the observed rate of raised anti-TPO antibodies between the cases and controls was statistically significant (P =.02).

In the 31 to 60-year-old age group, of the 32 (46.38%) cases, raised anti-TPO antibodies were found in 11 (34.38%) while among the 28 (40.58%) controls, raised anti-TPO antibodies were detected in 3 (10.71%) controls. A significantly higher rate of raised anti-TPO antibodies was noted in the cases as compared to the controls (P = .03; Table 2).

Regarding gender distribution, 13 (18.84%) cases and 11 (15.94%) controls were men. In men, a total of 4 (30.77%) cases were found with raised anti-thyroid peroxidase antibodies, while no control was found with raised anti-TPO antibodies. The difference was not statistically significant between cases and controls (P = .09). In the 56 (81.16%) cases involving women, anti-TPO antibodies were found to be raised in 16 (28.57%). Of 58 (84.06%) controls that were women, anti- TPO antibodies were found to be raised in 5 (8.62%). The difference was statistically significant (P = .00; Table 3).

DISCUSSION

Hypothyroidism is a very common pathological state of hormone deficiency that is potentially serious but eminently treatable (Biondi et al., 2008). Thyroid gland disorders are associated with morbidity stretching worldwide in almost 110 countries, and more prominently seen in the developing world (Khan et al., 2002). These disorders stay undetected because

Table 2: Comparison of elevated anti-thyroid peroxidase antibodies in both age groups

Group	Raised Anti-thyroid peroxidase antibodies		Total	*P*-value
	Yes (%)	No (%)		
18 to 30-year-old age group				
Cases	9 (24.32)	28 (75.68)	37 (53.62)	.02
Controls	2 (4.88)	39 (95.12)	41 (59.42)	
31 to 60-year-old age group				
Cases	11 (34.38)	21 (65.63)	32 (46.38)	.03
Controls	3 (10.71)	25 (89.86)	28 (40.58)	

Table 3: Comparison of elevated anti-thyroid peroxidase antibodies in men and women

Group	Raised anti-thyroid peroxidase antibodies		Total	*P*-value
	Yes (%)	No (%)		
Men				
Cases	4 (30.77)	9 (69.23)	13 (18.84)	.09
Controls	0	11 (100)	11 (15.94)	
Women				
Cases	16 (28.57)	40 (71.43)	56 (81.16)	.00
Controls	5 (8.62)	53 (91.38)	58 (84.06)	

clinical assessment alone is less specific and sensitive and can identify only up to 40% of the symptomatic cases. Only biochemical testing can confirm the diagnosis (Saha et al., 2007).

It has been established in many studies that higher prevalence rates of hypothyroidism are associated with advancing age and being a woman, particularly when thyroid autoantibodies are present (Canaris et al., 2000; Roberts CGP et al., 2004). Also, autoimmune thyroid disease is an organ-specific autoimmune disorder found usually in women between 30 to 50 years of age (Swain et al., 2005). Our results are consistent with these studies as 16 (28.57%) of 56 (81.16%) women with hypothyroidism were found to have raised anti-TPO antibodies. Also, in the 31 to 60-year-old age group, 11 (34.38%) of 32 (46.38%) patients with hypothyroidism showed the raised anti-TPO antibodies.

A study published by Carvalho et al. in 2013 showed that anti-TPO antibodies were detected in 90% to 95% of patients suffering from autoimmune thyroid disorders (AITD), were positive in 80% of patients with Graves' Disease, and 10% to 15% of non-AITD patients (Carvalho GA De et al., 2016). These results were consistent with those published by Mariotti et al. in 1990 (Mariotti et al., 1990). The literature also reported that patients of Hashimoto thyroiditis with very high anti-TPO antibody titers had persistent pathological concentrations of anti-TPO antibody despite the provision of adequate and appropriate medical treatment (Schmidt M et al., 2008). Furthermore, studies by Whickham and Busselton revealed that higher levels of serum TSH at

baseline escalated the chances of development of hypothyroidism, and also that this probability further soared with the existence of thyroid peroxidase antibodies in the serum (Hollowell et al., 1994; Walsh et al., 2010).

Several large-scale studies have established a high prevalence of the anti-TPO antibodies in euthyroid subjects, as well. For example, in the National Health and Nutrition Examination Survey (NHANES) III study, 11.3% of approximately 17,000 subjects without any known thyroid disorder had the presence of antibodies (Hollowell et al., 1994). The Whickham follow-up study of a US population also revealed a higher prevalence of anti-TPO antibodies in normal women (14.6%) and men (8.0%) (Vanderpump et al., 1995). Also, a study by Konno et al. in Japan found high titers of these autoantibodies in the disease-free Japanese population as well. Our study was also in agreement with these statistics and established that 7.25% of euthyroid subjects had raised anti-TPO antibodies.

For primary care environments, physicians need to be aware of this potentially high-risk demography so that patients can be tested for antibodies and monitored more closely. In our research, it was hypothesized that the expression of thyroid peroxidase antibodies could serve as an indicator of possible thyroid disease, and we intended this analysis to see raised anti-TPO antibodies in patients with hypothyroidism and euthyroid subjects so that some realistic suggestions could be put forward to prevent the development of hypothyroidism by early detection of anti-TPO antibodies and subsequent early management by immunosuppressive drugs. Although our hospital is a tertiary care public sector hospital and provides healthcare services to a large proportion of the population in the Bahawalpur district, the population presenting in this hospital is representative of the general population in this district only, and therefore, large scale studies encompassing larger areas should be carried out for better results.

CONCLUSIONS

There is a higher rate of raised anti-thyroid peroxidase antibodies in cases of hypothyroidism in contrast to euthyroid controls. Raised anti-TPO antibody was also significantly associated with age and female gender. The physicians should therefore, take into account the levels of these antibodies in the screening of the women especially above 30-years of age so that early disease management would notably have better outcome.

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Correlation of Serum Uric Acid Level with Blood Pressure in Middle-aged Population: A Cross-Sectional Study from Public Sector Hospital of Islamabad

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ABSTRACT

In Pakistan hypertension is the leading cause of mortality and morbidity and is affecting approximately one-third of the population, meanwhile hyperuricemia is also highly prevalent and has important clinical implications, but association of hypertension and hyperuricemia is still a matter of debate in Pakistani population. To determine the correlation between serum uric acid and Hypertension. Cross-sectional analytical study of six month duration was carried out at at tertiary care public sector hospital of Islamabad from February to August 2019. After taking the informed written consent 100 middle-aged (40 to 65years) asymptomatic individuals visiting tertiary care hospital for the test of serum uric acid for the first time were enrolled. Beckman Coulter AU analyzers and uriucase method was used to measure uric acid as per manufacturer's instructions. Blood pressure of all enrolled participants was measured through a mercury sphygmomanometer. Demographic information of the study participant, clinical history, height, weight, family history, uric acid level and blood pressure were recorded on pre-structured questionnaire. BMI was calculated by using WHO formula. Among 100 enrolled study participants, 50 (50%) were males and 50 (50%) were females. The mean age was 50±8 years. The frequency of hyperuricemia was 16.0% and among these 93.8% were hypertensive with a significant Pearson correlation coefficientp value of 0.014. There is a significat correlation between hyperuricemia and hypertension. Our findings remain to be confirmed in future prospective studies.

Keywords: Hyperuricemia, Systolic blood pressure, Diastolic blood pressure

INTRODUCTION

Globally Hypertension is the most common chronic diseases, affecting more than one billion people (Faris et al., 2017). In the United States, it is a significant health problem, with an estimated 65 million adults suffering from hypertension (Fields et al., 2004). Increased serum uric acid levels have been associated with hypertension (Ogbera and Azenabor., 2010), diabetes (Cappuccio et al., 1993), obesity (Nakanishi et al., 1999), insulin resistance (Dehghan et al., 2008), dyslipidemia (Bonora et al., 1996) and cardiovascular diseases. Regardless of many hypertension-related successes over the years, higher-than-preferred hypertension prevalence and lower-than-optimal BP control rates reflect a continuing need for active health policy and practice (Fields et al., 2004).

Hyperuricemia is a metabolic disturbance of purine nucleotide and considered a precursor of gout. Previous studies have examined the putative association between serum uric acid levels and blood pressure (Conen et al., 2004; Wingrove et al., 1998).

Literature from Pakistan is lacking regarding association between serum uric acid levels and raised blood pressure that is not only a cardiovascular risk factor but also plays a role in renal and metabolic diseases (Lu et al., 2009). Uric acid is the end product of purine metabolism in humans (Rashid et al., 2009). High plasma uric acid causes gout and is a risk factor for cardiovascular diseases (Kuzuya et al., 2002; Lara-Castro et al., 2007). Hyperuricemia occurs in 16% of cases dying due to any cause and in 39% due to cardiovascular disease (Lin et al., 2007).

In Pakistan as both the prevelance of hypertension and hyper Ureciemia are raising to alarming level, it is the required to determine if there is any association between hyperuricemia and hypertension in adult Pakistani population.

MATERIAL AND METHODS

It was a cross-sectional analytical study. Total of 100 middle-aged individuals 40 to 65 years of age were enrolled in visiting polyclinic laboratory for the investigation of their uric acid level first time. Their blood pressure level was measured through a mercury sphygmomanometer. Systolic blood pressure ≥130 mm Hg or Diastolic blood pressure ≥85 mm Hg was defined as Hypertension and serum uric acid concentration of >7mg/dL in men and ≥5.7mg/dL in women was labelled as hyperuricemia. Demographic information of the study participant (age, gender) clinical history, height, weight, uric acid level, blood pressure, family history of hypertension, hyperuricemia was recorded on a pre-structured questionnaire. BMI was calculated by using WHO formula.

Beckman Coulter AU analyzers and uriucase method was used in hospitals lab to measure uric acid as per manufacturer's instructions. Blood pressure was measured using a sphygmomanometer after resting for more than 5 min. Those with systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg were labelled as hypertensive (Bergler-Klein., 2019).

In order to keep patient information confidential, we used the codes only known to the researcher instead of their identifyable identities. We also used the aggregate information to present for public instead of individual information from tis limited group of patients.

The data was entered and analyzed by using SPSS version 20.0 The correlation between serum uric acid and blood pressure was determined by using the Pearson correlation coefficient. P-Value of ≤0.05 was considered statistically significant.

RESULTS

Total 100 study participants visiting tertiary care hospital were enrolled with a mean age of 50±8 years. Among them 50% were male and 50% were female. Overall 44% were literate and 56% were illiterate. The proportion of overweight was 48% while 23% were obese and only 26% of study participants were normal of normal weight. While 65% of the study participants have an elevated level of systolic blood pressure while 40% of the participants had an elevated level of diastolic blood pressure.

It is salient that males are more prone to hypertension. When we combine the systolic and diastolic blood pressure to estimate the overall hypertension status either systolic is elevated or both systolic and diastolic is raised, 71% of the patients were hypertensive. Among females, the frequency of hypertension is 66% while for males it was 76%. About 16% of the study patients were raised serum uric acid levels (Table 1).

Table 1: General Characteristics of the Study Participants

Variables		Gender		Total
		Male	Female	
		(n=50)	(n=50)	(n=100)
Education Status	**Literate**	19(38%)	25(50%)	44
	Illiterate	31(62%)	25(50%)	56
BMI	**Under weight**	2(4%)	1(2%)	3
	Normal	16(32%)	10(20%)	26
	Overweight	26(52%)	22(44%)	48
	Obese	6(12%)	17(34%)	23
Systolic Blood Pressure	**Raise**	33(66%)	32(64%)	65
	Normal	17(34%)	18(36%)	35
Diastolic Blood Pressure	**Raise**	27(54%)	13(26%)	40
	Normal	23(46%)	37(74%)	60
Uric Acid(mg/dL)	**Raise**	4(8%)	12(24%)	16
	Normal	46(92%)	38(76%)	84

By applying the Pearson correlation coefficient, it was found that systolic and diastolic blood pressure is correlated with serum uric acid at $p \leq 0.05$ while the strength of correlation between systolic blood pressure and serum uric acid is too weak i-e SBP vs SUA= 0.199, the same is for the diastolic blood pressure DBP vs SUA= 0.217. (Table 2 & Figure 1). Table 3 suggested that hyperuricemia is significantly associated with gender and hypertension.the odds of hyperurecemia for females are1.658 times higher than males. The odds of hyperurecemia in hypertensive patients are 1.406 times higher than normotensive. Although age and Education is not significantly associated with hyperuricemia but the odds of hyperurecemia for young adult (age less than 50 years) are higher than old adult. The odds of hyperurecemia in illiterate patients are 1.41 times higher than literate.

Table 2: Correlation of serum uric acid level with Blood Pressure

	Correlation Coefficient (r)	P-Value
Systolic Blood Pressure	.199*	0.047
Diastolic Blood Pressure	.217*	0.03
*. Correlation is significant at the 0.05 level (2-tailed).		
**. Correlation is significant at the 0.01 level (2-tailed).		

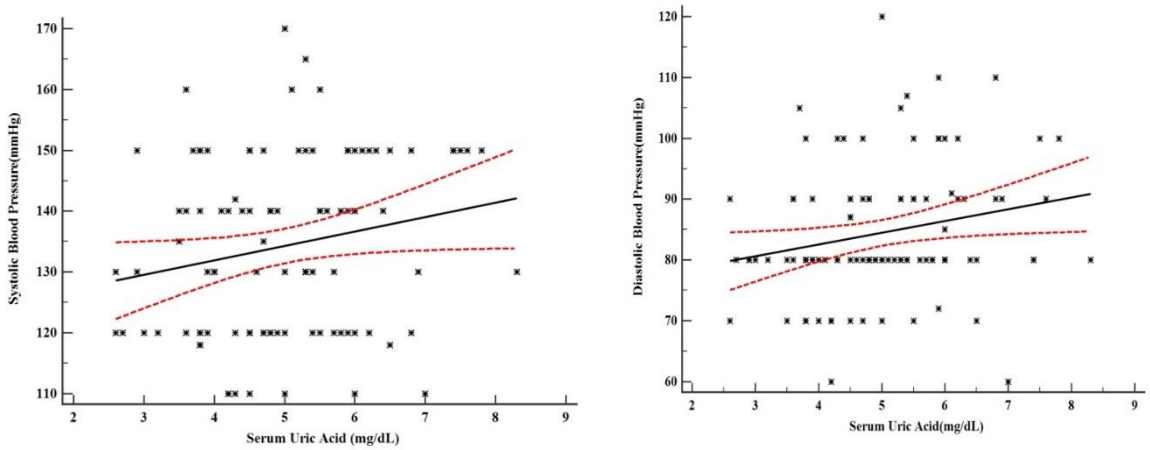

Figure 1: Corelation of serum uric acid level with systolic (left panel) and diastolic blood pressure (Right panel).

xshows scatter points of serum uric acid level and systolic and diastolic blood pressure level. The ▬ line was the linear regression line of the scatters in the plots. The red dash lines were the 95% Confidence interval of predicted mean. SBP, systolic blood pressure . SPSS, Statistical Package for the Social Sciences. SUA, Serum uric acid. WHO, World Health Organization. BMI, Body mass index

Table 3: Odd Ratios for hyperurecemia with multiple risk factors

Variables	Odd Ratio	95% confidence Interval	P-Value
Gender Female	1.658	1.148-2.395	0.026
Age<50	1.105	0.642-1.903	0.726
Education Illiterate	1.141	0.745-1.748	0.568
Obesity	2.365	0.754-7.420	0.133
Hypertension	1.406	1.155-1.713	0.029

DISCUSSION

The present study showed a 16% prevalence of hyperuricemia in adult population. This is almost in accordance with China 13.3%(36), Thailand 10.6% and Turkey 12.1%(37-38). Worldwide reported prevalence ranging from 2.6- 36 percent in different population (Uaratanawong et al., 2011).

The present study showed serum uric acid concentration were directly correlated with systolic and diastolic blood pressure. This positive relationship between Serum uric acid and hypertension has been described in numerous populations (Loeffler et al., 2012; Gois and Moraes Souza., 2017; Sundstrom et al., 2005). It is evident from Several emipdimologiacal studies that hyperure-cemia is accompanied with hypertension (Gois and Moraes Souza., 2017; Sundstrom et al., 2005; Yokokawa et al., 2016). It is salient to find that odds of hyperurecemia for hypertension 1.406 times higher. These findings were

congruent with Lee (Lee et al., 2015) they found 1.25 higher odds of hyperurecemia for hypertension C-I (1.08-1.45).

Our study reported that the odds of hyperurecemia for females are1.658 times higher then males. This gender difference in uric acid levels in favor of women is most probably due to female gonadal hormones. Uric acid increased with age in men and women, irrespective of body mass index (Kanjilal et al., 2008). Although uric acid increases with age, this increase occurs more in women, especially after menopause. In a Chinese study, hyperuricemia was noted more in post-menopausal women (Lu et al., 2009).

This study described that hyperurecemia is associated with young adults the odds of hyperurecemia for young adults are 1.105 times higer then elders .this is consistent with findings of Chinese adults aged 41 to 50 years (Lee et al., 2015). There is a dire need to explore exact mechanism for the age-related correlation between derum uric acid and hypertension in presence of other confounders (Pogodina et al., 2014; Scheepers et al., 2017). Odds of hyperuricemia for obesity found to be 2.365 higher than normal weight/lean. This is confirmed from a study on Bangladesh adults (Ali et al., 2018).

The results of the study can not be generalized due to certatin limitation firstly because of its design and sample size, secondly more confounder like fasting glucose, lipid parameters, dietry behavior physical activity,smoking status needs to be addressed. Further studies are needed to investigate the exact mechanism between serum uric acid and blood pressure.

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Cross talk of serum elements, cardiac and liver enzymes in patients with HCV chronic hepatitis and hepatocellular carcinoma in Pakistani population

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ABSTRACT

HCV-associated hepatic pathologies are now the frequent inducer of cardiac abnormalities because of cardio-hepatic interaction. Studies are going on to elucidate and highlight the possible factors involved in this complex interaction, but this paradigm is still unclear. Here, we aimed to explore the interrelationship among electrolytes, cardiac, and liver enzymes in HCV-associated hepatic abnormalities, including chronic hepatitis and hepatocellular carcinoma in the Pakistani population. 100 Hepatitis C virus (HCV) infected patients with liver disorders and 50 healthy individuals were recruited in the present analysis. Trace elements, ions, and enzyme concentrations were quantified *via* an automatic analyzer, while HCV was confirmed by enzyme-linked immunosorbent assay (ELISA). Our results demonstrated that serum Ca^{++}, Mg^{++}, Fe^{++}, Cl^{-}, and PO_4^{-} levels were significantly increased in HCV patients with hepatic pathologies, including cirrhosis and carcinoma. Cardiac enzymes, including aspartate aminotransferase (AST), creatine kinase (CK_2), and lactate dehydrogenase (LDH) concentration, were also elevated in HCV patients. Furthermore, serum cholesterol and triglyceride levels significantly differed in HCV patients than in normal individuals. Impaired alkaline transaminase (ALT) and alkaline phosphatase levels in HCV patients further validate the HCV patients' hepatic pathologies. Interestingly, all these impaired factors were positively correlated with the progression of hepatic disorders. In conclusion, altered ionic concentrations, cardiac enzymes, and liver dysfunction markers suggest their significant relationship to HCV leading liver pathologies in the Pakistani population.

Key Words: HCV, Electrolytes, Cardiac enzymes, Liver enzymes, Hepatic pathologies

INTRODUCTION

The primary cause of both acute and chronic liver disease is the Hepatitis C virus (HCV). Worldwide, up to 170 million people are chronically infected by HCV. 80% of the HCV-infected individuals become chronic carriers who may then progress to severe liver diseases. Within 2-3 decades of infection, 10–20% of chronically HCV-infected individuals develop severe liver cirrhosis, and 1–5% may develop hepatocellular carcinoma (HCC). HCV is a hepatotropic flavivirus in the Hepacivirus genus. It is a positive-stranded RNA virus with a genome size of 9.6 Kb (Chen & Morgan, 2006; Modi & Liang, 2008; Zaltron, Spinetti, Biasi, Baiguera, & Castelli, 2012).

HCV infections are associated with severe alterations of host body redox status. HCV infection can leads to oxidative stress through multiple mechanisms that include chronic inflammation, iron overload, and liver injury by increasing oxidative stress markers in some HCV patients (Paracha et al., 2013). The HCV induces liver damage accompanied by Fe^{++} overload-induced oxidative stress, inflammatory responses, cytotoxicity induced by virus core proteins, immune-mediated processes that show a significant relationship between HCV and endogenous elements. (Arain et al., 2014; Choi & Ou, 2006; Guo, Chen, Lin, Shih, & Ko, 2012; Rahman, 2007).

Iron (Fe) is an essential component of catalase enzymes, hemoglobin, and myoglobin. It also plays as a pro-oxidant and creates oxidative

stress in the presence of lipids. Individuals with high levels of lipids and serum iron are at increased risk of cancer. Iron accumulation, an outcome of viral and liver damage, is a common finding in chronic hepatitis C, but detailed mechanisms have not yet been fully elucidated (Berg et al., 2001; Kohgo, Ikuta, Ohtake, Torimoto, & Kato, 2007; Lambrecht et al., 2011; Nahon, Ganne-Carrié, Trinchet, & Beaugrand, 2010; Price & Kowdley, 2009). The high mutation rate of HCV and elevated liver enzyme ALT has been associated with the marked increase in Fe's levels in infected individuals (Rashed, 2011). Iron depletion maintained higher sustained virological response rates and significantly reduced ferritin levels and ALT activity (Franchini, Targher, Capra, Montagnana, & Lippi, 2008; Gentile et al., 2009; Tessman & Romani, 1998).

Similarly, alterations in cellular ions (i.e., Mg^{++}, Ca^{++}, Na^{+}, K^{+}) homeostasis influence biological membrane fluidity or produce reactive molecules (i.e., free radicals), which hamper the operation of signal transduction pathways in the liver, cardiac, and smooth muscle cells (Tessman & Romani, 1998). Exposure to toxic elements and alterations in essential elements homeostasis may be the risk factor for HCV infection (Lingala & Ghany, 2015). In the present study, the relationship of endogenous elements/electrolytes alteration is observed in the hepatic disorders common in the Pakistani population.

A condition termed cirrhotic cardiomyopathy, characterized by hyperdynamic circulation, increased cardiac output, reduced peripheral vascular resistance, and arterial pressure, represents the association between cirrhosis and cardiovascular abnormalities. Numerous cellular signaling pathways contribute to these abnormalities, including cardiovascular dysregulation, central nervous system, and humoral factors such as nitric oxide. Both endogenous and exogenous cannabinoids have significant cardiovascular effects. Certain evidence suggests that in cirrhosis at multiple levels, increased activity of the endocannabinoid system contributes to the development of both cardiac and vascular changes (Moezi, Gaskari, & Lee, 2008).

Previous studies have demonstrated liver and heart abnormalities' co-existence because both are systemic diseases (Xanthopoulos et al. 2019). Further elevated cardiac enzymes such as AST, LDH, and CK2 have been reported in different liver diseases (Neuschwander-Tetri 2017) but in Pakistani population it is essentially required to give some potential data showing interplay of cardiac, liver enzymes and essential elements in HCV pathologies. This study is designed to investigate this gap and to give some basic correlating data regarding altered levels of some ions, cardiac, and liver enzymes in liver diseases, specifically hepatitis C and hepatocellular carcinoma.

MATERIAL AND METHODS

Subjects

This is an observational and analytical study comprised of HCV patients with liver complications. Blood samples were collected from Benazir Bhutto Hospital (BBH) and Holy Family Hospital (HFH) Rawalpindi, Pakistan. A total of 150 subjects were selected (n =100, hepatitis with HCV patients, n = 50 were healthy individuals). Subjects were excluded if they were <20 or >80 of age. Prior informed consent was taken at the time of blood collection and questionnaire filling. The questionnaires were carefully filled, including personal data, past and present clinical history, family history (ultrasound and liver biopsy reports).

Ethical considerations

The present study was approved by the institutional ethical board and was conducted according to the Helsinki Agreement's guidelines.

Blood Sample Collection

Blood for biochemical and electrolyte analysis was collected in coagulant-containing gel tubes and immediately stored at -4 °C. The serum

was then separated as supernatant from cells by centrifugation at 4000 RPM for 5 minutes and stored at -20 °C until analysis.

Diagnosis of HCV through ELISA

HCV was diagnosed by the Diagnostic kit for Antibody to Hepatitis C Virus (ELISA) for the qualitative detection of antibodies to

hepatitis C in human serum as previously described (Afridi et al., 2014)

Biochemical Determinations

The concentration of liver enzymes, i.e., alanine aminotransferase (ALT), alkaline phosphatase (Arain et al.), and Cardiac enzymes, i.e., aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK_2), were determined in serum by the Enzymatic rate method with SYNCHRON CX9-PRO automatic analyzer (Beckmann Coulter, USA) using commercially available assay kits. Serum total bilirubin levels were determined by a timed endpoint diazo method in SYNCHRON CX9-PRO automated analyzer (Beckmann Coulter, USA) using a commercially available assay kit.

Electrolytes Determination:

Serum electrolytes i.e., Na^+, K^+, Cl^-, Ca^{++}, Mg^+, $Fe^{++,}$ and PO_4^- were determined using Ion-Selective Electrode (ISE) with SYNCHRON CX9 PRO automatic analyzer (Beckmann Coulter, USA). Manufacturer's instructions with commercially available Synchron buffers and reagents were utilized. An ISE (Ion-selective electrode), made of glass or validamycin, is a thin membrane with selective binding sites across which only the specific ion can be transported from higher to a lower concentration and creating a potential difference through binding these sites. The sample was diluted with a high ionic strength ISE electrolyte buffer, minimizing the variation in the specimens' activity coefficients to be analyzed. The ISE electrolyte buffer and ISE electrolyte reference maintain ion activity constant on the electrodes. A potential was generated at the surface of ISE when the diluted sample passes through the flow cell. The magnitude of potential change was proportional to the concentration of the respective electrolyte. Nernst equation was used to determine the concentration of electrolyte from this potential (Albert, Subramanian, Rangarajan, & Pandey, 2011).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) post by Turkey's multiple comparison test and Student's t-test using SPSS 16. All the data presented as mean ± SD 16. $p < 0.05$ was considered to be significant.

RESULTS

Impaired cardiac enzymes level correlates with HCV-associated hepatic abnormalities

In the present analysis 100 patients (HCV hepatitis) and 50 healthy control individuals were included (patient: age above 20 years, male: female 62: 48, Control age above 20 years, male: female 38: 12). The final diagnosis patients were: HCV + Chronic (n = 68), HCV + Cirrhosis hepatitis (n = 32).

After HCV validation in the patients with liver disorders, including chronic cirrhosis and carcinoma, cardiac enzymes (CK_2, AST, and HDL) were assessed in the serum of under observational subjects (Table 1). Interestingly, cardiac enzymes level was significantly higher in HCV patients with hepatic abnormalities than the control subject (Table 1). Further statistical analysis demonstrated that CK_2, LHD, and AST levels were significantly higher in patients with HCV-chronic conditions; however, in HCV-with cirrhosis patients, LHD and AST levels were significantly higher compared to the control subjects (Table 2). This finding suggests that HCV-associated hepatic pathologies could impair cardiac enzymes status and activities.

Table 1: Comparison of electrolytes, cardiac and liver enzymes between liver patients and healthy individuals

Parameters	Patients (n = 100)	Controls (n = 50)	P values
Cardiac enzyme			
CK2 (IU/L)	155.6 ± 221.10	85.4 ± 32.18	0.0273
LDH (IU/L)	323.6 ± 163.60	133.88 ± 26.80	<0.0001
AST (IU/L)	82.9 ± 8.3	25.8 ± 10.18	<0.0001
Electrolytes			
Ca++ (mg/dl)	9.54 ± 0.51	8.9816 ± 0.629	<0.0001
Mg^{++}(mg/dl)	1.95 ± 0.40	0.7102 ± 0.26	<0.0001
Fe^{++}(ug/dl)	163.7 ± 56	90.755 ± 296.8	<0.0001
Na+ (mmol/l)	135.5 ± 6.8	138.76 ± 3.579	0.007
K+(mmol/l)	3.6 ± 0.66	4.39 ± 0.62	<0.0001
Cl- (mmol/l)	108.9 ± 10.9	101.67 ± 5.28	<0.0001
PO4-- (mg/dl)	3.95 ± 0.79	3.42 ± 0.524	0.002
Liver enzymes			
T.bil (mg/dl)	2.52 ± 3.18	0.5673 ± 0.309	<0.0001
ALT (IU/L)	181.80 ± 32.5	29.580 ± 11.86	<0.0001
ALP (IU/L)	251.1 ± 169.7	184.2 ± 64.2	0.046
Lipid profile			
Cholesterol (mg/dl)	148.7 ± 103.7	165.98 ± 21.6	<0.0001
Triglyceride (mg/dl)	211.7 ± 154.5	100.67 ± 35.5	<0.0001

Values are given as means ± SD. p values were calculated by using the Student's t-test.

Table 2: Multiple comparison of cardiac enzymes among chronic (n = 68), cirrhosis (n = 32) and normal (n = 50) subjects.

Parameters	CK2 (IU/L)	LHD (IU/L)	AST (IU/L)
Chronic+ HCV	169.1 ± 265.7	322.4 ±176.2	80.5 ± 68.3
Normal	85.4 ± 32.2	133.4 ± 26.7	25.8 ± 10.0
P values	0.0390	<0.0001	<0.0001
Cirrhosis+ HCV	126.9 ± 55.2	326.5 ±136.6	88.1± 109.8
Normal	85.4 ± 32.2	133.4 ± 26.7	25.8 ± 10.0
P values	0.5739	<0.0001	<0.0001

Values are given as means ± SD. *p* considered significant when less than 0.05**.**

HCV infection accelerates hepatoxicity

Retarded functioning of liver enzymes and alterations in their specified expression is reported in the liver abnormalities (Tolleson,

2018). Herein to validate the liver toxicity, we measured liver toxicity markers, including total bilirubin, ALT, and ALP. As expected, altered levels of liver enzymes were detected in the serum of patients with hepatic pathologies compared to control subjects (Table 1). Notably, all the proteins mentioned above (ALT, ALP, and total bilirubin) were significantly impaired in the serum of the HCV patients with chronic hepatic pathology conditions. In contrast, only total bilirubin differed considerably in HCV with cirrhosis patients than normal individuals; however, we did not detect any significant change in ALT and ALP levels (Table 3).

Table 3: Multiple comparisons of liver enzymes and lipids level among chronic, cirrhosis, and normal subjects

Parameters	**liver enzymes (IU/L)**			**Lipids (mg/dl)**	
	Total bilirubin	ALP	ALT	Cholesterols	Triglycerides
Chronic + HCV	2.36 ± 2.59	263.7 ±170.7	190.9 ±348.6	144.3 ±79.74	211.8 ±149.5
Normal	0.56 ± 0.306	184.7 ±64.2	29.8 ± 11.9	165.7 ±21.45	100.3 ± 35.81
P values	0.001	0.01	0.005	0.54	<0.0001
Cirrhosis+ HCV	2.85 ± 4.21	224.7 ±164.9	162.8 ±275.9	158.2 ±143.1	211.5 ± 167.2
Normal	0.56 ±0.306	184.7 ±64.2	29.8 ± 11.9	165.7 ±21.45	100.3 ± 35.81
p values	0.001	0.61	0.08	1.00	0.001

P values were calculated by ANOVA post by Turkey's test. Values are given as means ± SD.

Serum electrolytes/elements status in HCV-associated hepatitis

Determination of the level of the electrolytes in the serum of patients and control subjects was carried out. Interestingly, we found an impaired electrolytes level between HCV patients and control subjects (Table 1). Ca^{++}, Mg^{++}, Fe^{++}, Cl^{-} and PO4 were significantly higher in HCV infected patients than in healthy individuals. However, serum K^{+} concentration was significantly decreased in HCV patients (Table 4). These findings may suggest the contribution of specific electrolytes/elements in HCV and liver disorder

Cholesterols and triglyceride

The liver is an essential organ for lipid metabolism, and an impaired lipid profile could contribute to the progression of hepatic pathologies (Luo, Pu, Wang, & Xu, 2010). Herein, we found a significantly altered level of both cholesterol and triglycerides in patients' serum compared to the normal subjects (Table 1). Cholesterol level (144.3 ± 79.7) was significantly decreased in HCV chronic conditions compared to normal (165.7 ± 21.45). In contrast, the triglyceride levels were significantly increased in both HCV with chronic (211.8 ± 149.5) as well as in cirrhosis (211.5 ± 167.2) liver abnormalities compared to normal (100.3 ± 35.81) subjects (Table 3). The results may indicate the deregulation of lipids in HCV-induced liver damage conditions.

DISCUSSION

Here, we demonstrated that HCV infection accelerates liver toxicity by increasing hepatotoxicity markers impairment. HCV

Table 4: Electrolytes concentration among HCV chronic, cirrhosis, and normal individuals

Electrolytes	Normal	HCV + Chronic	p values
		HCV + cirrhosis	
Ca^{++} (mg/dl)	8.9920 ± 0.62754	9.5353 ± 0.51421	<0.0001
		9.5594 ± 0.53028	<0.0001
Mg^{++} (mg/dl)	0.7120 ± 0.26927	2.0029 ± 0.42985	<0.0001
		1.9156 ± 0.34837	<0.0001
Fe^{++} (ug/dl)	90.4000 ± 29.4881	154.75 ± 55.6023	<0.0001
		180.91 ± 55.75138	<0.0001
Na^{+} (mmol/l)	138.70 ± 3.5642	135.56 ± 6.8423	0.016
		136.69 ± 6.81761	0.411
K^{+} (mmol/l)	4.3940 ± 0.62316	3.6838 ± 0.72824	<0.0001
		3.7281 ± 0.52685	<0.0001
Cl^{-} (mmol/l)	101.62 ± 5.24459	108.01 ± 10.07360	0.001
		111.06 ± 10.58586	<0.0001
$PO4^{--}$ (mg/dl)	3.4240 ± 0.52121	3.8868 ± 0.78072	0.002
		4.0969 ± 0.82050	<0.0001

Values are given as means ± SD. *p* considered significant when less than 0.05

infection could also enhance cardiac enzymes during liver cirrhosis condition, supporting the cardio-hepatic interaction. Impaired lipid profile (cholesterol and triglycerides) supports this notion. Furthermore, HCV-associated hepatitis aggravates the imbalance in electrolytes concentration, which may further contribute to the dysregulation of the biological process involved in the hepatic pathologies.

Hepatitis C virus (HCV) infection is the major risk factor that can lead to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Lingala & Ghany, 2015). Enhanced AST activity, LDH, and CK_2 are general cardiac pathologies markers reported in specific hepatic pathologies (Neuschwander-Tetri, 2017). Additional parameters analysis in the coexisted liver and cardiac pathologies have also been carried out in other populations (Xanthopoulos et al., 2019). However, the expression and mechanism of serum up-regulation of CK_2 and its isoforms are still under discussion. The elevated CK_2 level has also been reported as a functional contributor in chronic HCV hepatitis (Anderson, Zeng, Rock, & Yoshida, 2000; Faloppi et al., 2014). In the present study, a significant (p = <0.0001) increase in cardiac enzyme levels in HCV patients, specifically with chronic hepatitis compared to healthy individuals, suggests an interrelationship of cardiac enzymes and HCV. The upregulated level of dysfunctional liver markers, including bilirubin, ALP, and ALT, confirmed liver abnormalities in agreement with previously documented studies (Wahib, Seif El Nasr, Mangoud, El Shazly, & Morsy, 2005).

Electrolytes play essential roles in various biological processes, and their homeostasis is critical for life (Bertini & Cavallaro, 2008). The ions act as cofactors such as iron, an integral component of catalase enzymes, hemoglobin, and myoglobin (Kohgo et al., 2007; Nahon et al., 2010). It is also well known that the high mutation rates of HCV and raised liver enzyme ALT levels have been associated with markedly increased Fe^{++} in infected individuals (Gentile et al., 2009). Fe^{++} ions in NADPH's presence can also induce Ca^{++} ions from the liver microsome damaging Ca^{++} and Mg^{++} ATPase (Rolfs & Hediger, 1999). In the present study, we detected a significant up-regulation of ions, including Fe^{++}, $Ca^{++,}$ and $Mg^{++,}$ in patients diagnosed with HCV hepatitis. The absence of K^+ and LDH release in the perforated or the extracellular compartment suggests that the release of Mg^{++} induced by ethanol occurs through the operation of a specific transport process (Onji et al., 1992). A significant decrease in the K^+ ion concentration in sera of the patients has suggested its role and is in agreement with previous findings. Under the umbrella of the mentioned shreds of evidence, the current findings may present a significant link between altered ionic concentrations in HCV and liver abnormalities.

CONCLUSION

In summary, we conclude that cardiac enzymes (CK_2, AST, and LDH), electrolytes/elements, liver enzymes (ALT and ALP), and total bilirubin deregulation play a significant role in HCV-allied chronic hepatitis as well as in cirrhosis carcinoma. This study also suggests a relation between electrolytes, lipids, cardiac and liver enzymes in hepatitis. They may hold the key to understanding the unique biological roles of these factors in different liver disorders and malignant transformation.

Declarations

Ethical Approval and Consent to participate : Yes

Consent for publication. Yes

Availability of data and materials. All data generated or analyzed during this study are included in this article.

Competing interests: The authors declare no competing financial interests.

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Association between Grain Size, Shape and Thousand Kernel Weight in Pakistani Wheat Landraces

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ABSTRACT

Wheat (*Triticum aestivum*. L) grain size is considered to be one of the main criteria of yield constituents by wheat breeders. In order to detect phenotypic diversity and relationship between thousand kernel weight, a collection of 204 landraces from different parts of Pakistan was planted across two years (2012-2014). High throughput method based on seed imaging was used to measure the kernel size and shape. The correlation analysis revealed significant positive correlation between thousand kernel weight (TKW) with perimeter of vertical (PV), thickness (T), area of vertical (AV), area of horizontal (AH), perimeter of horizontal (PH), width (W) and Length (L). Bioplot showed that accessions with high seed shape parameters have higher TKW. By taking TKW as a dependent variable multiple regression analysis was performed. Regression summary indicated that 31% of the variations in TKW are explained by the independent variables. It was shown that grain thickness, length and width are most important for predicting TKW. Based on image analysis this study provides useful information about the relationship between TKW, kernel size and shape in Pakistani wheat landraces that may help to improve grain weight in a breeding program.

Keywords: Landraces, Wheat, Seed morphology, thousand kernel weight

INTRODUCTION

In Pakistan wheat remains the major staple food. Average yearly wheat production is almost equal to 24.032 million tons to 9.046 million hectares (Oury and Godin., 2007). Increase in population puts a pressure for demand of increased yield of wheat. The current cultivars lack genetic diversity making them an easy target for many biotic and abiotic stresses (Hussain et al., 2011). In contrast with modern wheat varieties, primitive wheat species exhibit wide variations in grain size, shape and quality parameters due to uniform selection of these traits in elite wheat cultivars (Gegas et al., 2010). Because of wide variations in phenological, morphological, abiotic, biotic and quality traits landraces and other wild species play a major role in breeding programs (Moragues et al., 2007 ; Moragues et al., 2006).

Important agronomic traits like seed shape and size affect the yield and market price. The seeds of a single plant have very few differences in their shape and size, therefore

large amount of measurements are needed to obtain accurate size data (Tanabata et al., 2012). Digital imaging generates the measure of length, width, perimeter and area which are used to define dimensions. It can also capture seed roughness, asymmetric skewing and thickness that contribute to shape variations (Williams et al., 2013). Studies based on grain morphology can be used to exploit increasingly sophisticated phenotyping methods. In wheat such measurements can be related to yield or milling qualities. These traits are economically significant but expensive to measure. Based on geometric models it is recommended that wheat kernel shape and size might effect flour yield as spherical seeds have the maximum possible endosperm to bran ratio (Marshall et al., 1984). Grain weight by volume is the primary criteria used to grade wheat before milling, and is found to be associated with wheat kernel size and shape (Campbell et al., 1999). Harlan (Harlan., 1975) described wheat grain as a major focus of assortment since the dawn of agriculture. Grain weight is a complex quantitative and polygenic trait, affected by numerous heritable interactions at all stages of its growth and development. Grain weight is a very stable yield component and is positively related with agronomic yield (Golan et al., 2015).

Plant breeders are aggressively following the new quick and accurate methods of phenotyping to benefit from the lower price genotyping processes (de Souza., 2010 ; Houle et al., 2010 ; Montes et al., 2007). Computerized digital imaging is a non-destructive and non-invasive technique that can acquire, process and analyze data attained from images (Richardson et al., 2001; Díaz-Lago et al., 2003; Karcher et al., 2003). Improvement of photometric processes or digital imaging will offer more accurate, cheaper phenotypic information to better explain the individual component of complex traits. The digital imaging process generates quantitative data from digital images of plant organs (Kwack et al., 2005 ; Dana and Ivo., 2008 ; Diao et al., 1999). Digital imaging has the capacity to determine the dimensions of seed morphology that eventually contribute to grain weight.

Grain size is one of the major components of the domestication syndrome in cereals (Fuller., 2007). Evidences from the fertile crescent indicated that the transition from the diploid einkorn and tetraploid emmer wheat to the domesticated forms was associated with a trend towards large grain size (Fuller., 2007 ; Feldman., 2000). Grain size and shape also effect complex traits like thousand kernel weight (TKW) (Zhang et al., 2014). Kernel shape becomes important in current time due to market preferences and industrial demands (Gegas et al., 2010). Grain size is largely described by grain weight and area, whereas shape means the comparative proportions of the major growth axis of the grains (Breseghello and Sorrells., 2007 ; Gegas et al., 2010). It was observed in many studies that grain size and shape have a positive correlation with TKW and have influenced four yield, end-use quality and market price (Evers et al., 1999 ; Breseghello and Sorells., 2006 ; Tsilo et al., 2010 ; Cui et al., 2011 ; Blanco et al., 2012 ; Williams and Sorells., 2014 , Rasheed et al., 2014). TKW is a complex trait and is controlled by many traits like grain shape and size (Zhang et al., 2014). Generally length, width, sphericity and horizontal axes proportions and vertical perimeters determine the grain shape (Breseghello and Sorrells., 2007). Grain weight and area are used to characterize grain size (Gegas et al. 2010, Breseghello and Sorrells., 2007).

Image analysis has been implicated for the grain examination (Davies et al., 2003) damaged kernel identification (Luo et al., 1999) differentiation of grains of different species (Chtioui et al., 1996) and determination of flour milling yield potentials in wheat (Berman et al., 1996).

Plant domestication and the beginning of an agricultural centered economy initiated the most important human cultural development. Highly conscious selection has taken place in the transfer of plants from their wild habitats to a new human managed environment (Abbo et al., 2009, Abbo et al., 2011 ; Abbo et al.,2014). The process of domestication has led to many morphological and physiological changes, most of which are related to domestication process (Olsen and Wendel., 2013). Gegas *et al*. (Gegas et al., 2010) carried out examination of grain weight and shape of ancestral wheat species and elite cultivars and observed that elite varieties with heavy grains had kernels that were wider and shorter than those of wild *Triticum* species. Due to laborious and time consuming techniques the exact characterization of grain size and shape remains a huge challenge (Houle et al., 2010 ; Rasheed et al., 2014; Patil et al., 2013) recently used high throughput methods to capture grain size and shape. In spite of the importance of the relationship between grain morphology and TGW a lack of information is evident especially on primitive cultivars like landraces. This study was undertaken to realize the importance and the need of such study on hexaploid wheat landraces. Therefore the evaluation of grain shape, size and TKW was carried out on a set of 204 landraces collected from different areas of Pakistan.

MATERIALS AND METHODS

Seeds from 204 landraces collected from different parts of Pakistan were used in this study. Seeds were acquired from the gene bank of Plant Genetic Resources Institute (PGRI), National Agricultural Research Center (NARC) Islamabad. The genotypes were planted in field conditions during two growing seasons, i.e. 2012-2013 and 2013-2014 in National Agricultural Research Center (NARC) Islamabad (33° 33' N and 73° 06'E). Seeds were hand threshed and 25 sound seeds from each accession were selected for imaging. Seeds were positioned horizontally and vertically on a black paper at an equal distance. For horizontal images the major axis of seeds defined grain length and minor axis related to grain width. While in case of vertical images the major axis corresponded to grain width and minor axis to thickness. A standard measuring 1 cm^2 was placed with seeds while taking photographs. The photographs taken were termed according to the genotype accession number and planting year. *Smart Grain* developed by the National Institute of Agro biological Sciences, Tsukuba, Ibaraki, Japan, was used to measure multiple seed perimeters in addition to the area. *Smart grain* automatically recognizes all the seeds in a digital image and then calculates area of horizontal (AH), area of vertical (AV), perimeter of horizontal (PH), perimeter of vertical (PV), length (L), width (W), thickness (T), length to width ratio (LWR), circularity of horizontal (CSH), circularity of vertical (CSV), distance of center of gravity from the vertical intersection of length and width (DSV) and horizontal intersection of length and width (DSH) Statistical analysis (descriptive stats, PCA and multiple regression analysis) was performed with the software XLSTAT 2010. Multiple regression analysis was performed by the exclusion of the variables in high inter-correlation.

RESULTS

Manual methods of measuring grain morphology have some limitations due to the number of data, quality of measured characteristics and different gleaned shape data. Whereas computational methods like digital imaging help in generating data associated with seed size and shape in a small time interval (Williams et al., 2013). Grain shape and size are important agronomic traits along with grain quality due to their significant influence on grain weight, milling yield, end use quality and market value (Abdipour et al., 2016).

Analysis of variance revealed significant differences among genotypes for AH, AV, PH, PV, L, T and TKW while there was significant difference (P<0.001) between years for all investigated traits. Analysis of variance showed that there are no significant interaction between genotype and year for any traits which means genotypes respond to year variation in same ways (Table 1).

Table 1: **Mixed linear model applied, with genotype as fixed and years as random effects. Mean squares of studied traits of 204 genotypes across two years.**

SOV	df	AH	AV	PH	PV	L	W	T
Genotypes (G)	203	5.46*	3.11***	2.00***	1.365*	0.28***	0.06ns	0.08***
Year (Y)	1	1233.7***	764.9***	740.35***	523.03***	40.39***	19.7***	35.01***
GXY	203	4.29ns	2.07ns	1.45ns	1.02ns	0.18ns	0.06ns	0.05ns
		LWR	**CSH**	**CSV**	**DSH**	**DSV**	**TKW**	
Genotypes (G)	203	0.019ns	0.001ns	0.0007ns	0.007ns	0.001ns	97.22***	
Year (Y)	1	0.38***	0.29***	0.2***	0.004ns	0.082***	7.07***	
GXY	203	0.016ns	0.0009ns	0.0008ns	0.006ns	0.001ns	0.263ns	

Significance *** = p-value ≤ 0.001; * = p-value ≤ 0.05

Digital image analysis provided the information about the shape dimensions. Statistical description (Table 2) showed the pattern of parameters with respect to range, central tendency (mean) and level of dispersion (standard deviation, SD) along with a coefficient of variation percentage. Thousand kernel weight is one of the key yield associated trait. TKW in these landraces ranged from 14.6 to 48.84g in 2013 and 16.12 to 49.4g in 2014. Mueen-ud-Din *et al*. (Mueen-ud-Din et al., 2007) reported the range of 39.22 to 43.16g in Pakistani wheat varieties. Anjum *et al.* (Anjum et al., 1998) observed thousand kernel weight ranging from 31.4 to 37.2g. Variations in TKW are because of environment and genetic control (Halverson et al., 1988). Grain length ranged from 5.9 to 10.5mm in 2013 and 5.5 to 9.5mm in 2014. Grain width ranged from 3.1 to 4.5mm in 2013 and 2.7 to 4.1mm in 2014. Butt *et al*. (Butt et al., 1997) reported the grain length of 5.06-7.01mm and width of 5.79-7.12mm width. Maximum and minimum variability were observed in the seed area (horizontal) during the year 2013 and vertical circularity in the year 2014. Dispersion to mean ratio expressed, as coefficient of variation (CV %) was highest in DS horizontal and for vertical seed area during 2013. High range of TKW was observed during both years expanding, coefficient of variation percentage.

Correlation among traits is important in plant breeding, due to its reflection in dependence degree between different traits. Both genetics and environmental factors influence the phenotypic correlation among traits. The correlation analysis revealed significant positive correlation between TKW with PV, T, AV, AH, PH, W and L (Table 3). This suggests that by increasing one of them the other trait is increased. Abdipour (Abdipour et al., 2016) reported that several grain size and shape measure-

Table 2: Statistical description of studied parameters.

Statistic	AH (mm^2)		AV (mm^2)		PH (mm)		PV (mm)	
	2013	2014	2013	2014	2013	2014	2013	2014
Minimum	13.077	11.936	6.944	6.613	16.530	14.785	11.065	10.556
Maximum	29.148	25.046	15.870	13.468	29.088	23.851	18.175	15.266
Range	16.070	13.110	8.926	6.855	12.558	9.066	7.110	4.710
Mean	20.082	16.604	11.867	9.128	20.386	17.692	14.756	12.492
SD	2.337	2.072	1.914	1.233	1.449	1.188	1.314	0.814
CV %	11.638	12.482	16.132	13.502	7.106	6.717	8.904	6.515
	L (mm)		**W (mm)**		**T (mm)**		**LWR**	
Minimum	5.943	5.535	3.106	2.784	2.826	2.643	1.611	1.609
Maximum	10.515	9.551	4.560	4.195	4.356	3.823	2.644	3.009
Range	4.573	4.016	1.454	1.411	1.530	1.179	1.033	1.400
Mean	7.092	6.462	3.844	3.404	3.691	3.105	1.858	1.919
SD	0.484	0.485	0.284	0.227	0.311	0.219	0.140	0.130
CV %	6.827	7.504	7.379	6.671	8.425	7.059	7.511	6.779
	CSH		**CSV**		**DSH (mm)**		**DSV (mm)**	
Minimum	0.435	0.490	0.596	0.626	0.310	0.331	0.286	0.245
Maximum	0.685	0.719	0.749	0.778	0.862	0.786	0.515	0.499
Range	0.250	0.230	0.153	0.151	0.552	0.456	0.229	0.253
Mean	0.608	0.662	0.681	0.726	0.506	0.500	0.379	0.351
SD	0.037	0.026	0.034	0.022	0.095	0.076	0.044	0.043
CV %	6.078	3.899	4.940	3.040	18.743	15.221	11.530	12.229
	TKW (g)							
Minimum	14.6	16.120						
Maximum	48.84	49.420						
Range	34.24	33.300						
Mean	32.25	33.110						
SD	7.02	7.230						
CV %	21.76	22						

ments like length versus aspect ratio, area and perimeter and thickness versus width are inherently correlated. According to a study conducted, (Rasheed et al., 2014) grain length and width were positively correlated with volume and perimeter. Seeds that are larger in size have the ability to produce vigorous seedlings. Seed size is positively associated with seed strength. Drikvand (Drikvand et al., 2013) and Ramya (Ramya et al., 2010) reported a positive correlation between thousand kernel weight grain length and width.

Principal component analysis (PCA) is a classical technique applied widely for analyzing data, compression and data set's

features visualization. PCA revealed the relationship among studied variables along with their importance towards capturing the variability. Importance of the shape parameters is inversely proportional to the magnitude of angle adapted with PC1 and PC2 respectively. In this regard, width (W), thickness (T) and length (L) were selected in the first dimension (PC1) and length to width ratio (LWR), CSH (circularity of horizontal) and DS (distance of center of gravity from the intersection of length and width) were selected from the second dimension (PC2).

Table 3: Pearson's correlation coefficient (r) of studied traits

Traits	AH	AV	PH	PV	L	W	T	LWR	CSH	CSV	DSH	DSV
AV	**0.645**											
PH	**0.910**	**0.532**										
PV	**0.636**	**0.966**	**0.539**									
L	**0.881**	**0.429**	**0.867**	**0.444**								
W	**0.840**	**0.738**	**0.714**	**0.698**	**0.520**							
T	**0.621**	**0.975**	**0.527**	**0.948**	**0.399**	**0.732**						
LWR	0.110	-0.254	0.236	-0.204	**0.550**	**-0.420**	**-0.277**					
CSH	-0.163	0.057	**-0.421**	-0.006	**-0.444**	0.124	0.060	**-0.608**				
CSV	0.056	0.159	0.002	-0.087	-0.037	0.181	0.164	-0.205	0.232			
DSH	0.225	-0.029	**0.336**	-0.003	**0.378**	-0.048	-0.016	**0.460**	**-0.297**	-0.062		
DSV	**0.318**	**0.331**	0.258	**0.352**	**0.321**	0.210	0.263	0.132	-0.077	-0.074	0.169	
TKW	**0.459**	**0.490**	**0.395**	**0.492**	**0.370**	**0.388**	**0.492**	0.003	-0.014	0.058	0.112	0.265

*.Values in bold are different from 0 with a significance level alpha= 0.0001

Area and perimeter were also found close to X-axis with significantly highest squared cosines on PC1 dimension. Only vertical circularity was found to the third dimension. About 65.56 % of variation was explained within two dimensions (PC1 and PC2). TKW having significant squared cosine along PC1 indicated that it has surely been explained in the same direction as length, width and thickness (Table 4).

Genotypes with serial no. 9 (accession no. 12021: location Mansehra), 120 (accession no. 18873: location Chitral), 119 (accession no. 12233: location Abbottabad), 133 (accession no. 18776: location Abbottabad) and 177 (accession no. 11549: location Balochistan) made the periphery of the Biplot indicating the extremes. Genotype 9 (accession no. 12021: location Mansehra) and 19 (accession no. 12104 : location Muzaffarabad) were with high PV, W, T and TKW (Figure 1). Genotype 120 (accession no. 18873 : location Chitral) and 119 (accession no. 12233: location Abbottabad) were best in seed length and horizontal parameter. Accessions spread in overall left half of the Biplot were lower in all the shape parameters. Seed roundness (circularity) was best in genotype no. 135 (accession no. 12016: location Abbottabad), 95 (accession no. 18667 : location Swat) and 168 (accession no.

Table 4: Principal component analysis (PCA) illustrating the diversity captured by studied variables.

	PC1	PC2	PC3			
Eigenvalue	5.694	2.829	1.082			
Variability (%)	43.799	21.761	8.325			
Cumulative %	43.799	65.560	73.885	PC1	PC2	PC3
	Eigenvectors			**Squared cosines**		
AH	0.384	0.091	0.206	**0.839**	0.023	0.046
AV	0.364	-0.218	-0.118	**0.755**	0.135	0.015
PH	0.359	0.246	0.154	**0.734**	0.171	0.026
PV	0.362	-0.170	-0.306	**0.747**	0.081	0.101
L	0.325	0.324	0.178	**0.602**	0.298	0.034
W	0.350	-0.199	0.205	**0.698**	0.112	0.045
T	0.356	-0.228	-0.097	**0.721**	0.148	0.010
LWR	0.005	0.540	-0.006	0.000	**0.823**	0.000
CSH	-0.074	-0.447	0.064	0.031	**0.565**	0.004
CSV	0.026	-0.194	0.750	0.004	0.106	**0.609**
DSH	0.077	0.349	0.091	0.034	**0.344**	0.009
DSV	0.178	0.080	-0.396	0.181	0.018	0.170
TKW	0.248	-0.034	-0.108	**0.350**	0.003	0.013

11229 : location Balochistan) and the same genotypes are lowest in LWR (Figure 1). In the Biplot, genotypes in the right half from the extreme right are good in all shape parameters. The Biplot explained that the accessions with high values of seed shape parameters also possess high TKW (Figure 1) for example, genotype no. 9 (accession no. 12021: location Mansehra), 19 (accession no. 12104: location Muzaffarabad), 120 (accession no. 18873: location Chitral), 20 (accession no. 12114: location Balochistan), 12 (accession no. 11931: location Northern Areas), 106 (accession no. 11766: Skardu), 8 (accession no. 18842: location Dir), 6 (accession no. 12012: location Abbottabad). TKW in various studies was observed to be positively correlated with agronomic yield (Fuller., 2007 ; Cui et al., 2011 ; Maccaferri et al., 2010) and flour yield (Williams and Sorrells., 2014 ; Breseghello and Sorrells., 2006 ; Chastain et al., 1995).

Multiple regression analysis was performed by taking TKW as dependent Y-variable. Model ANOVA (Table 5-A) showed the significance of the hypothesis. Ratio of model mean square with error mean square indicated that the predictors are appropriate to depict the values of TKW. Significant prediction of TKW has been assessed by the ANOVA table regarding independent estimates used in the model. Durbin-Watson d-value (1.9) is rejecting any possibility of autocorrelation among the predictors. Moreover the value of serial correlation (0.04) highlights that there is no auto-correlation between variables. This indicated that the most effective variables

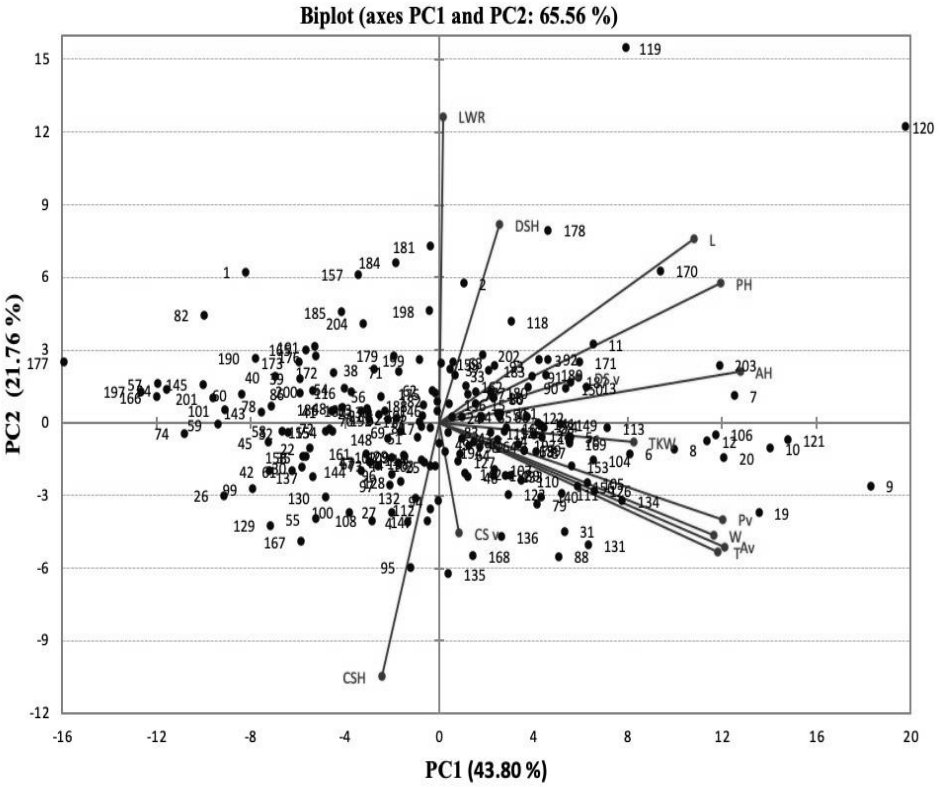

Figure 1: Biplot of studied variables along with the spread of 204 genotypes.

have been chosen for the prediction of TKW. Among 12 studied shape parameters, dependent six variables of the model were selected with the help of PCA and multicolinarity diagnostics. To avoid the multicolinarity, area and perimeters were not selected in the model because these were the function of length and width (included in the model). The selected six variables were the seed shape parameters with significant cosines at PC1 and PC2, included as the predictors of TKW (Table 5-B). Regression summary indicated that 31% of the TKW variation has been explained by the six independent variables. Regression equation of the model can be written as:

$$TKW = 87.549+24.718\ (L) - 41.86\ (W) + 15.5\ (T) - 74.04\ (LWR) + 19.69\ (CSH) + 7.133\ (DSH)$$

Relationship showed that thickness is the most important predictor to assess TKW (Table 5-C). Length and width are also important for the TKW prediction. Four out of six shape parameters significantly predicted TKW. Except few values, both (+0.95% and -0.95%) sides of confidence interval has been observed, hence revealing the prediction confidence for TKW with the help of shape parameters (Figure 2).

DISCUSSION

Image analysis is an important technique that can have many applications in identification of varieties and seed certification. For the crop improvement seed size and shape are important due to their influence on yield and quality. One of the major approaches to increase wheat yield is through the improvement of kernel weight. The variability in seed size and shape parameters provides initial

information to breeders to produce combinations in their programs for enhancing grain size in the new cultivars.

The correlation between seed parameters helps in suggesting that this correlation is related to uniformity and smoothness of kernel to grain weight ratio. The association of major seed dimensions also reveals that thickness of grain has maximum direct effect on grain weight which is followed by vertical area. It can also be revealed that horizontal area of seed has less impact on grain weight. Overall, this high throughput phenomic characterization identifies the underlying genetic mechanisms for grain size and shape at much more high resolution than that expected by conventional and manual seed measurement techniques. In a study by Gegas and colleagues (Gegas et al., 2010) on six wheat populations, it was confirmed that seed size and shape are highly independent traits. The genetic correlations which are estimated and measured here are consistent with previous observations in winter wheat (Dholakia et al., 2003).

It is observed in previous studies that length of seed is set earlier in developmental process whereas width of seed requires time to be influenced by environmental conditions during seed filling period (Sardras and Egli., 2008).

Table 5: **Regression analysis of shape parameters as independent X-variables and TKW as dependent Y-variable.**

A	SOV	DF	MS	F	Pr > F			
	Model	6	501.716	14.138	< 0.0001			
	Error	197	35.487					
	Total	203	(Computed against model Y = Mean (Y))					
B	SOV	DF	MS (I)	MS(III)	F (I)	F (III)	Pr > F (I)	Pr > F (III)
	L	1	1373.187	241.846	38.696	6.815	< 0.0001	0.010
	W	1	526.964	178.364	14.850	5.026	0.000	0.026
	T	1	874.107	964.346	24.632	27.175	< 0.0001	< 0.0001
	LWR	1	178.528	160.818	5.031	4.532	0.026	0.035
	CSH	1	26.336	25.409	0.742	0.716	0.390	0.398
	DSH	1	31.177	31.177	0.879	0.879	0.350	0.350
C	Variables	Beta	SE	T	Pr > |t|	-95%	95%	
	Intercept	87.549	72.466	1.208	0.228	-55.359	230.457	
	L	24.718	9.468	2.611	0.010	6.046	43.390	
	W	-41.865	18.673	-2.242	0.026	-78.690	-5.039	
	T	15.500	2.973	5.213	< 0.0001	9.637	21.364	
	LWR	-74.044	34.782	-2.129	0.035	-142.637	-5.451	
	CSH	19.697	23.278	0.846	0.398	-26.209	65.603	
	DSH	7.133	7.610	0.937	0.350	-7.874	22.140	

a) Over all variance analysis of the model of multiple linear regression.
b) Analysis of variance (Type I and III) for dependent variables.
c) Model parameters indicating Beta and t-values along with their probability.

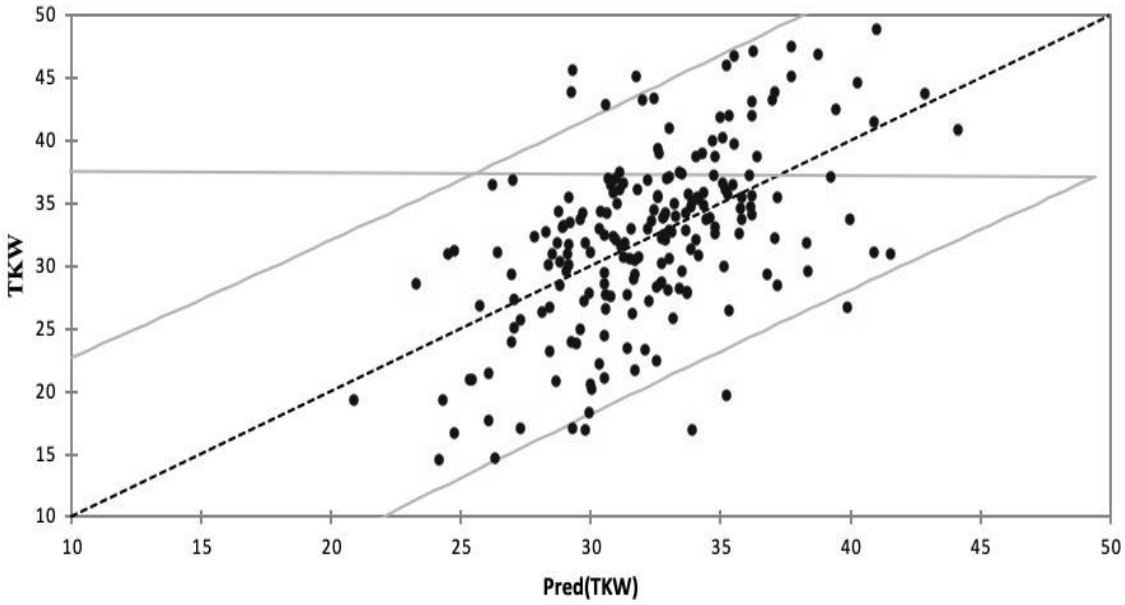

Figure 2: Scatterplot of TKW against predicted scores of TKW.

Our results have been in correlation with Rasheed (Rasheed et al., 2014), who reported a strong correlation between kernel and seed weight and size. Studies have shown that kernel weight has positive correlation with grain yield and kernel growth rate are less correlated with grain yield across environments (Ramya et al., 2010).

Knowledge of seed shape and morphological characters is required for direct manipulation of seed morphology to improve quality and yield in wheat. Low correlations and negative correlations for seed characters show that these characters should be manipulated independently. Similarly, positive correlating characters should be manipulated together (Abdipour et al., 2016).

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REVIEW ARTICLE

A Comprehensive View on Cardiovascular Diseases (CVDs): Genetics, Risk Factors & Preventions

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ABSTRACT

Cardiovascular Diseases (CVDs) are one of the foremost causes of deaths across the world. This review aims to evaluate the genetics and risk factors involved in CVDs and to assess the preventive measures which can be taken for diminishing the chances of developing CVDs. The goal of this review is to provide researchers and clinicians dealing with vascular disorders with a compendium of data about the genetic causes, risk factors, and preventive strategies to combat the development of CVDs. We searched online databases including PubMed for peer-reviewed scientific papers, case studies and review articles related to CVDs, emphasizing on the role of genetics and risk factors like diabetes, hypertension, smoking, alcohol consumption, obesity, age & gender in the progression of CVDs, and reviewing the role of diet and exercise in the prevention of CVDs. Managing the risk factors involved in CVDs is the most essential step for the inhibition of vascular diseases. Healthy lifestyle interventions consisting of a well-balanced diet and physical activity are very critical for the prevention of CVDs. Trials carried out on model organisms have indicated a direct link between diet and exercise on cardiovascular conditions. Strategies involved in the treatment of vascular diseases should also include low-fat diet plans like consumption of whole grains, fruits, vegetables, yogurts and avoiding high-saturated fat-containing foods with the addition of performing moderate aerobic exercises including cycling, swimming, hiking, and running to eliminate the root of the problem.

Keywords: Cardiovascular Diseases (CVDs), Genetics, Cardiovascular Risk Factors (CRFs), Preventive Measures, Diet & Exercise, Model Organ

INTRODUCTION

Cardiovascular diseases (CVDs) are termed as disorders of the heart and blood vessels. A number of these heart disorders are caused due to the lack of physical activity and inadequate diet. Some genetic factors also play a key role in developing numerous heart complications. CVDs have become the leading cause of premature deaths in countries across the globe. Many risk factors are involved in increasing the chances of evolving vascular disorders. Some of the major risk factors include (1) Diabetes, (2) Hypertension, (3) Smoking, (4) Alcohol Consumption, (5) Obesity, (6) and Age & Gender. CVDs can sometimes be classified based on their causing factors. Vascular disorders can be caused due to partial or complete blockage of blood vessels. For example, Thrombosis (Formation of the clotted mass of blood within a vessel). Some of the widely occurring heart disorders include Atherosclerosis, Angina Pectoris, Myocardial Infarction (Heart attack), etc.

In addition to the commonly known CVDs, there is a different class of heart problems known as congenital heart diseases (CHDs), heart disorders that are produced in fetal life and occur as birth defects. The majority of these are caused due to genetic mutations. For example, patent ductus arteriosus (PDA), is a birth defect in which the ductus arteriosus fails to close after birth causing the mixing of oxygenated and deoxygenated blood. Infants with this disorder are referred to as Blue Babies.

Hypertension is caused due to high blood pressure. Factors that are a basis for high blood pressure also lead to many occurring CVDs. Various treatments are present for curing different CVDs. In the case of most of the heart disorders, surgeries are performed, and drugs are administered for treatment. Broadly used methods for surgical treatment include angioplasty, coronary bypass, and open-heart surgery.

The increase in CVDs in the past few years has been linked to an unhealthy lifestyle, physical inactivity, high cholesterol levels, and high blood pressure. The risk factors involved are directly contributing to causing CVDs. Genetics also plays a key role in susceptibility to major risk factors. As the treatments involved in curing CVDs are long, time-consuming, and costly processes, it would be easier to understand the risk factors involved and take appropriate measures for the prevention of heart diseases. Without taking in to account the causative factors, effective treatment cannot be developed for any disease. CVDs are one of the leading causes of deaths worldwide, so developing preventative measures against them is a major step forward towards saving countless lives.

Genetics: Influence on Cardiac conditions

Inherited cardiac conditions (ICC) are a sub-type of CVDs caused by genetic changes including SNPs, mutations, and epigenetic change (Care et al., 2017). A Variety of studies have indicated a link between CVDs and genetic conditions (Moser., 1985).

The diagnosis of heart disorders and stroke varies in people of different ethnic backgrounds. People belonging to Asian backgrounds are at twice the risk of developing CVDs as compared to their European counterparts (Aambo & Klemsdal., 2017; Martin., 2018). Genetic variations occurring genes involved in autoimmune pathways, can eventually lead to vascular disorders (Perrotti et al., 2017).

In the case of congenital heart diseases (CHDs), four different categories of genes have been identified which lead to abnormal heart conditions (Arcelli et al., 2010). About 400 genes are linked to causing CHDs (Jin et al., 2017). Approximately 35% of congenital heart diseases in patients are caused due to genetic factors (Simmons & Brueckner., 2017). Many of the existing CHDs occur in African nations like Rwanda, where genetic defects are very common (Teteli et al., 2014). Transcription factors and their enhancers are also involved in causing CHDs. Overexpression of transcription factors (*TBX5, NKX2-5,* and *GATA4*), and their enhancers (*MYH6 & NPPA*) has been shown to cause the defect in drosophila (Amodio et al., 2012).

Table 1 shows a list of Genes and their effects related to CHDs occurring in humans. Studying the effects of these genes affiliated with the occurrence of CHDs can help design novel therapies for the diagnosis and treatment of vascular disorders.

Table 1: Genes associated with the development of Congenital heart diseases (CHDs).

Genes	OMIM ID	Normal Function	Resulting Defects	References
SMAD3	603109	Intracellular signals transducer	Increase risk of ventricular septal defects (VSDs)	(Li et al., 2015b)
ACTA2, FBN1 TGFBR2	102620, 134797, 190182	Protein formation	Bicuspid aortic valve disease	(Giusti et al.,2017)
TBX20 CASZ1	606061, 609895	Transcriptional regulation	Dilated cardiomyopathy	(Kennedy et al., 2017)
LEFTY1 LEFTY2	603037, 601877	Left-right Asymmetry during development	Increase risk of CHDs	(Deng et al., 2014)
TCN2	613441	Transport proteins	Increase risk of CHDs	(Li et al., 2017)
MTHFR, MTRR	607093, 602568	Amino acid synthesis	Increases risk of VSDs	(Noori et al., 2017)
FOXC1	601090	Embryonic development	Multiple CHDs	(Du et al., 2016)
ALPK3	617608	Cellular differentiation	Primary cardiomyopathy	(Çağlayan et al., 2017)
ZW10	603954	Chromosomal segregation	Associated with CHDs	(Sun et al., 2018)
GATA4, JAG1, FOXC2, TBX5, TBX1	600576, 601920, 602402, 601620, 602054	Transcriptional factors and developmental regulation	Multiple CHDs including Conotruncal defects (CTDs)	(Morgenthau et al., 2018; Zhang et al., 2018)
MESP1	608689	Transcriptional proteins	Increases risk of VSDs	(Zhang et al.,2017)
MSX1 MSX2	142983, 123101	Transcriptional regulation	Associated with causing VSDs	(Li et al., 2015a)
NKX2-5	600584	Transcriptional factors	Impaired cardiomyogenesis	(Anderson et al., 2018)

Genetic mutations lead to complete heart failures (HF) in patients with ventricular disorders such as right ventricular apical (RVA) and atrioventricular block (AVB). Mutation in *LMNA* and *SCN5A* genes increases apoptotic rate, leading to the onset of early heart failure in (AVB) patients (Liu et al., 2017).

Coronary artery diseases (CADs) are among the most widely spread heart-related disorders. They are influenced by environmental as well as genetic components. Studies have shown a relation

between *MPO* gene and CADs. Therefore, people with single nucleotide polymorphisms in *MPO* gene are at significantly greater risk of developing CADs. Similarly, individuals with *IL-1beta* allele are more prone, towards CADs (Sreekanth et al., 2016; Arslan et al., 2017). Point mutations in *ADD1* and *ACE* genes have been observed in causing arterial hypertension (Cieslewicz & Jablecka., 2010).

Apolipoprotein E (Apo E) is involved in maintaining cholesterol levels. Decreased activity of the transcription factor phosphatase 1G reduces ApoE levels, which leads to several vascular disorders (Benson et al., 2017).

NO-cGMP Pathway Regulation

The Nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway (Figure 1) plays an imperative role in maintaining blood pressure, normal cardiac function, and cardiovascular homeostasis in the human body.

Nitric oxide (NO) is produced by endothelial nitric oxide synthase (eNOS) in the vascular endothelial cells of the arteries. NO eases blood flow and regulates vascular function by promoting vasodilation of blood vessels (Grassi et al., 2013). When increased blood flow is detected by the receptors due to any exogenous signal, nitric oxide is produced by eNOS in the endothelial cells which is then transferred to the nearby smooth muscle cells, in which NO acts as an intracellular signal. NO contributes towards the enzyme guanylyl cyclase as it converts GTP to cyclic GMP, resulting in smooth muscle relaxation and thus, contributing toward lowering the blood pressure. NO also protects the veracity of the endothelium and regulates vascular homeostasis by inhibiting leuko - cyte adhesion, proliferation, vascular inflammation, platelet adhesion, and aggregation of vascular smooth muscle cells (Forstermann & Sessa., 2012).

The NO-cGMP pathway is regulated by several genes (Table 2). Single nucleotide polymorphisms (SNPs) in corresponding genes like *ARG1*, *NOS3*, *GUCY1A3* and *PRKG1* results in NO deficiency by reducing eNOS activity, this eventually leads to the occurrence of numerous CVDs (Leineweber et al., 2017).

Cardiovascular Risk Factors (CRFs)

Diabetes

The coronary risk associated with diabetes increases the chances of developing coronary heart disorders (Davis et al., 2014). Type 2 diabetes mellitus (T2DM) is linked with many vascular disorders including coronary artery diseases (CADs), congestive heart failure (CHF), and myocardial infarction (MI). Due to genetic factors, poor glycemic control, and metabolic degradation, the chances of complete heart block (CHB) increases in diabetes patients (Agarwal & Singh., 2017; Martin., 2017a). The mortality rate increases in type 1 and type 2 diabetic patients with atherosclerosis caused due to the development of CVDs. A strong link between insulin resistance and atherosclerosis has been observed which is caused by ineffective insulin receptor (IR) signaling. Using inhibitors like (Trodusquemine) to reduce the activity of protein tyrosine phosphatase 1B (TP1B), which is a major regulator of IR, has shown prevention of plaque formation and reversal of atherosclerosis (Thompson et al., 2017). Whole-body glucose uptake as a response to insulin is termed as meal-induced insulin sensitization (MIIS). Insulin induces the release of hepatic insulin sensitizing substance (HISS) causing uptake of glucose in kidneys, heart, and skeletal muscles.

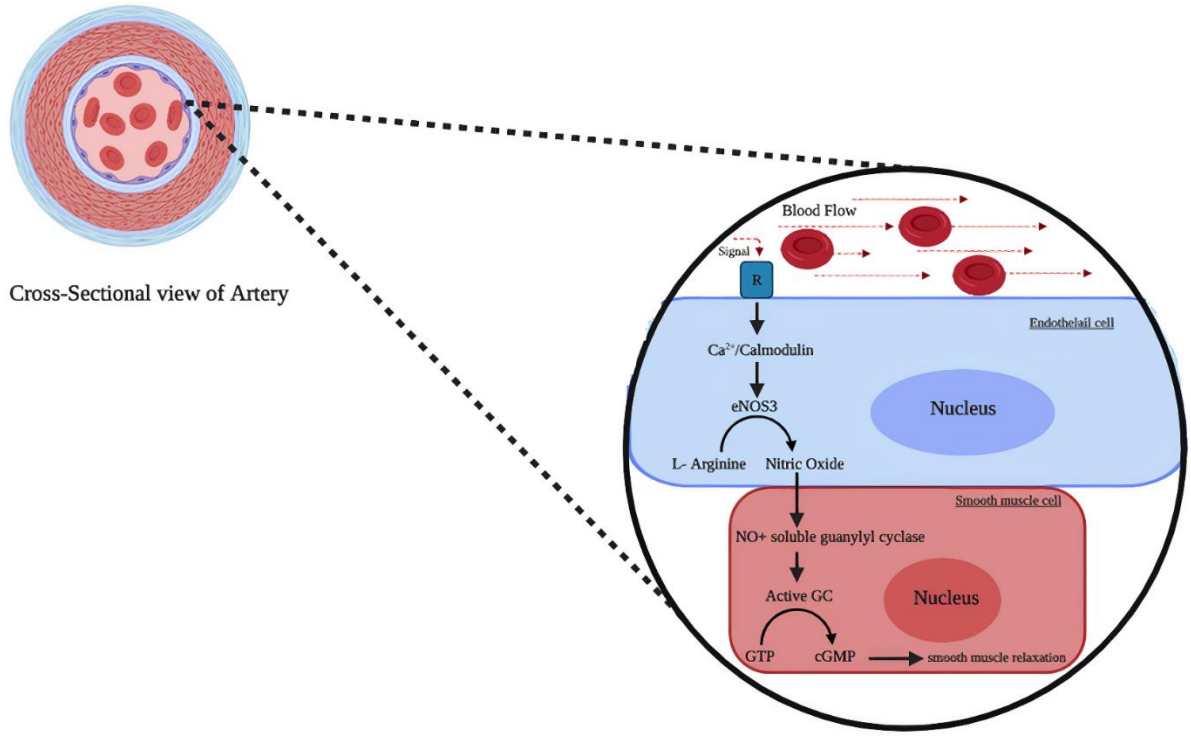

Figure 1: NO-cGMP Pathway: eNOS= endothelial nitric oxide synthase; R= receptor; NO= nitric oxide; GC= guanylyl cyclase; GTP= guanosine triphosphate; cGMP= guanosine monophosphate

hyperglycemia and hyperinsulinemia which leads to vascular dysfunction and cardiac problems (Chowdhury et al., 2013).

Diabetes has also been observed to be caused by postprandial blood glucose concentration in middle-aged women (45-50 years). moderate level of a walk after a meal can help decrease blood glucose level (Nygaard et al., 2009).

Although CVDs are correlated with diabetes and vice versa, the risk of developing type 2 diabetes in patients suffering from cardiac abnormalities is 2-4 times higher than in the general population (Kristjansson et al., 2015). Type 2 diabetes can be prevented by up to 28-59% by lifestyle changes like performing physical activities taking a low-fat diet and reducing mental and physiological stress (Walker et al., 2010; Martin., 2017b).

Red cell distribution width (RCDW) serves as a marker for detecting CVDs in patients with diabetes (Al-Kindi et al., 2017). Identifying diabetes in patients is the first step towards treatment, some amino acids like isoleucine, phenylalanine, and tyrosine show metabolic signatures which can help predict diabetes development in patients. Studies indicate aromatic and branched-chain amino acids as efficient markers for detecting susceptibility to CVDs in diabetic patients (Magnusson et al., 2013).

HYPERTENSION

Anxiety, depression, and mental trauma lead to stress. Individuals belonging to different ages, ethnic backgrounds, and genders are affected differently by stress. Genetic variations caused by stress can lead

Table 2: Genes involved in the NO-cGMP Pathway

Gene	OMIM ID	Normal Function	Resulting Defects	References
ARG1	608313	Urea Cycle/ Arginine synthesis	Myocardial Infarction	(Mónica et al., 2016)
ARG2	107830	Urea Cycle/ Arginine synthesis	Myocardial Infarction	(Zhang et al., 2019)
GCH1	600225	GTP cyclohydrolase I Synthesis	Hypertension	(Guo et al., 2017)
NOS3	163729	Nitric oxide synthesis	Myocardial Infarction,	(Bogdan, 2015)
DDAH1	604743	Methylarginine levels / Inhibits NOS	Hypertension	(Xu et al., 2017)
DDAH2	604744	Methylarginine levels/ Inhibits NOS	Hypertension	(Klinger & Kadowitz, 2017)
GUCY1A2	601244	GTP conversion	Hypertension, Coronary artery disease	(Wobst et al., 2018)
GUCY1A3	139396	NO receptor	Coronary artery disease	(Kessler et al., 2019)
PRKG1	176894	Smooth muscle relaxation	Hypertension	(Han et al., 2019)
PDE5A	603310	Hydrolysis of cyclic nucleotides	Hypertension	(Ueda et al., 2019)
NPPA	108780	Cardiac homeostasis	Heart failure, Hypertension	(Menon et al., 2019)
NPR3	108962	Vascular homeostasis, Fluid balance	Hypertension	(Gao, 2017)

to CVDs (Albert et al., 2017). Salt intake is important for preventing hypertension and vascular diseases (Ando et al., 2013). In the U.S, the increase in mortality rates due to heart failure, stroke, and coronary artery diseases are higher in the African American population than in the White population, because African Americans are more prevalent towards hypertension due to greater stress (Carnethon et al., 2017).

Major depression disorders (MDDs) are linked to patients suffering from coronary heart diseases. Depression and vascular disorders including many risk factors have led to the 'Vascular Depression' Hypothesis (Iosifescu et al., 2005).

Depression disorders are associated with an increased level of tumor necrotic factor-alpha and interleukin-6. It has been observed that higher levels of C-reactive proteins (CRP) are present in patients with MDDs, and elevated CRP levels are inter-related with CVDs. Genetic factors may also influence CRP levels (Kozlowski et al., 2006).

Post-traumatic stress disorder (PTSD) is often shown in CVD patients. Increased cytokines and homocysteine levels usually occur in (PTSD) (Brown et al., 2009; Goldstein et al., 2015; Sagud et al., 2017; Sawchuk et al., 2005). About 34% of adults in the U.S suffer from hypertension (Kones & Rumana., 2014). The major cause of death in pregnant women in the U.S is due to CVDs caused by physiological stress. About 33% of death in the U.S occur due to vascular disorders (Graves & Davis., 2018).

Smoking

Majorly occurring disorders including cancers are associated with smoking. The risk of liver, lungs, and colorectal cancer increases with smoking. An estimated 85% of lung cancer occurs in smokers (Sealock & Sharma., 2018). Many CVDs are linked to smoking. Irregular patterns of cardiac rhythms are often observed in smokers. Although smoking affects every individual differently, people who suffer from bipolar disorder (BP-1), schizophrenia and severe mental disorder (SMD) are more prone towards the development of hypertension and vascular disorder due to smoking (Birkenaes et al., 2007; Foguet Boreu et al., 2013; Goldstein et al., 2009).

Young adults (aged 18 to 30 years) and people belonging to South Asian ethnicity are at higher risk of developing early Atherosclerotic Cardiovascular diseases (ASCVD) due to many risk factors including smoking (Gooding et al., 2017; Kandula et al., 2015; Jelwan et al., 2020).

Smoking eventually leads to cancer, high blood pressure, vasoconstriction, coronary heart diseases, and organ damage. Patients with chronic kidney diseases (CKDs) are 10 times more likely to die of vascular disorders correlated to risk factors like smoking (Gregg & Hedayati., 2018). Although many individuals do know about the detrimental effects of smoking, very few take appropriate steps to quit smoking. A study carried out on patients with CVDs to investigate that if they were aware of the risk factors involved with vascular disorders indicated that 30% of the patients identified smoking as a leading risk factor (Montinaro et al., 2008).

Alcohol

Alcohol consumption has toxic effects on the heart and vascular system. It has multiple synergistic and synchronous consequences on the human body. Excessive alcohol intake causes dilated cardiomyopathy, induces arrhythmias, decrease myocardial contractibility, which leads to cardiovascular dysfunction, and causes tissue damage. Prolonged consumption of alcohol increases the risk of HF, hypertension, and CADs (Fernandez-Sola., 2015). Coronary atherosclerosis is often seen in patients with alcoholic cirrhosis (Danielsen et al., 2018).

Atrial fibrillation (AF), one of the most widely occurring forms of cardiac arrhythmias is interlinked with alcohol consumption and some other risk factors (Naser et al., 2017). The chances of developing CVDs increase with the increased ingestion of alcohol from light, moderate to heavy intake (Goel et al., 2018). Excessive alcohol consumption

(>14 units/week) increases the risk of mortality (Luksiene et al., 2017; Saito et al., 2018).

Alcohol consumption is among the three leading causes of premature deaths in the U.S, just behind smoking, and obesity. The risk of premature deaths is higher in males aged (15-49 years) due to alcohol abuse than it is in females of the same age group (O'Keefe et al., 2018; Flora & Nayak., 2019).

Different types of alcoholic beverages have different effects on the vascular system, increasing the risk of CVDs.

Obesity

The increase in chronic heart diseases around the world is directly related to the rise in obesity rates in developing countries, especially in U.S. About one-fifth of the children in the U.S and two-third of the adults in the U.S are overweight or obese.

Recent studies have shown that 33% of the U.S population is obese (Allison., 2017; Khan et al., 2009). The risk of developing CVDs rises in overweight individuals (BMI >/=25kg/m2) or higher, than in individuals with normal body weight (Eguchi et al., 2014). Although excessive eating is associated with obesity, some genetic factors that also influence a person's (BMI) (Goodarzi., 2018).

Obesity is a major lifestyle-based health complication (Lappalainen et al., 2014). Self-care is required for the prevention of obesity, which includes maintaining ideal body weight, and taking an adequate amount of nutrients (Frohlich & Al-Sarraf., 2013; Riegel et al., 2017). CVDs caused by obesity are one of the major causes of unnatural death among the population (Slockers et al., 2018).

Age and Gender

The risk factors intricated with vascular disorders have different effects on males and females belonging to different ages. The mortality rate due to ST-elevated myocardial infarction (STEMI) is twice in elderly females (>=65 years) than it is in elderly males (Juhan et al., 2018). Similarly, the effect of stress is higher on the elderly population as compared to the younger generations (O'Neil et al., 2018; Martin., 2016). The susceptibility to high blood pressure, hypertension, diabetes mellitus, and CVDs is also greater in aged individuals.

Prevention strategies against CVDs

Role of Diet

While considering the preventative measures which can be taken against CVDs, it is very

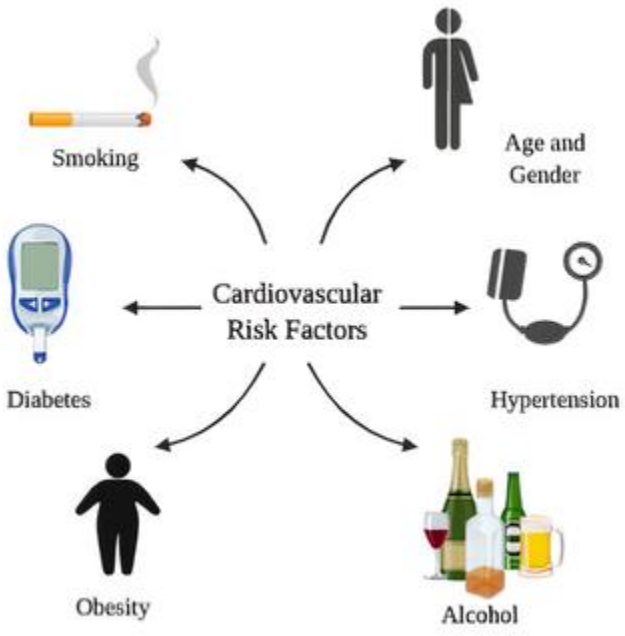

Figure 2: Systematics representation of risk factors involved in cardiovascular disorders

essential to address the root of the problem. An appropriate diet is the first step involved in the prevention process (Franklin et al., 2014). For the promotion of cardiovascular health, diet is vital to maintaining a healthy lifestyle by reducing the intake of saturated fats and increasing consumption of fresh fruits and vegetables (Anderson., 2018; Pouya et al., 2017).

It has been observed that foods that are rich in high anti-oxidants, like fresh fruits and vegetables, help reduce the risk of developing CVDs (van der Bom et al., 2002). Diet is considered the most significant factor while addressing the risks involved in CVDs. An adequate diet is associated with maintaining normal blood pressure and preventing ischemic heart diseases (IHD) (Arentoft et al., 2018). Taking the proper diet and adopting a healthy lifestyle greatly reduces the risk of Atherosclerotic cardiovascular diseases (ASCVD) in adults (Booth et al., 2016).

Excessive intake of high-fat containing foods leads to obesity, which results in hypertriglyceridemia, high blood pressure, and elevated cholesterol level. Ingestion of foods that are low in unsaturated fats can help prevent many coronary heart diseases (CHDs). Reducing the level of lipoprotein in the body also reduces the risk of developing CHDs. Patients with hypercholesterolemia, hypertension and diabetes mellitus are at greater risk towards the development of CHDs due to penurious eating habits. After the age of 40, the chances of developing CHDs in such patients increases 25% every 10 years.

A diet containing a moderate level of low-density lipoprotein (LDL) and decreased level of cholesterol can help prevent hypercholesterolemia (Simoons & Casparie., 1998). Refined sugar, starch, and carbohydrates in western diet have fatalistic effect on health and increase the risk of CHDs, while unsaturated fats have beneficial impacts on metabolic health and reduce the risk of CHDs (Willett., 2008).

Consumption of dairy products such as cheese and yogurt having anti-inflammatory properties, high nutritional value. and low-fat content can help prevent vascular diseases and have a positive effect on health (Lordan et al., 2018).

The risk of developing age-related CVDs greatly increases with a decrease in leukocyte telomere length (LTL). It serves as a marker for detecting cellular aging. Studies have indicated a link between consumption of processed red meat with shortening of LTL thus, contributing towards the early onset of development of age-related diseases (Fretts et al., 2016).

Foods like whole grains, fish oils, beans, walnuts, and almonds are also crucial for healthy vascular system. Flavonoids present in cocoa and dark chocolates have efficacious effects on cardiovascular health and display a cardioprotective role. Chocolate intake (1-5+ times/week) has shown to reduce the risks of developing CHDs (Djousse et al., 2011). Flavonoids intake in the form of fruits and vegetables reduces the risk of CVDs (Jacques et al., 2015; Wang et al., 2014).

Role of Exercise

Reducing body mass is an essential step involved in the preventative process. The risk associated with developing vascular disorders rises with obesity. Physical activity programs are recommended along with healthy diet plans to decrease obesity and lower the risk of developing CVDs (Pate et al., 2015).

The risk of coronary heart diseases (CHDs) increases with physical inactivity. Exercise helps maintain a normal blood glucose level and has positive impacts on cardiovascular conditions. Daily exercise can prevent several health-related issues (Opie & Dalby., 2014). Although the risk of developing CHDs varies from person to person, the effect of physical inactivity combined with poor diet and other risk factors are more prominent in peoples belonging to South Asian backgrounds (Arjunan et al., 2013; Fernandez et al., 2014). Moderate exercises like (jogging) improve endothelial functions in arteries, protecting vascular systems from the detrimental effects of a high-fat diet (Bond et al., 2015).

Studies have shown that aerobic and resistance exercises (like running, swimming, and hiking) can reduce abdominal obesity in women, reducing the risk of atherosclerosis (Choo et al., 2014). Cycling, walking, sports, and other physical activities can help reduce the risk of CHDs in older individuals (Koolhaas et al., 2016). Self-care and self-management through exercises are important for the prevention of heart failure (HF) (Lee et al., 2009). Physical activities, lifestyle modifications, and good nutrition can help prevent many CVDs. Taking a low-fat diet combined with aerobic exercises reduces BMI, decreases LDL levels and increases HDL levels, which also lowers the risk of developing atherosclerotic cardiovascular diseases (ASCVD) (Cugnetto et al., 2008; Wenger., 2014).

Training exercises and physical activities can help in weight loss (~2kg), preventing obesity and improving health (Swift et al., 2014). Continuous physical activities and aerobic exercises like cycling, hiking, swimming, and running are recommended for cardiac rehabilitation patients as they increase ventricular function and improve vascular health (Bjarnason-Wehrens et al., 2004; de Gregorio., 2018).

Some epigenetic changes like DNA methylation can be caused by performing physical activities, which play a vital role in the prevention of CVDs (Recchioni et al., 2017). The effects of exercise on an individual to reduce the risk of developing CVDs have been studied in numerous trials. Results show that (55-65 minutes) exercise performed at the rate of 4-5 times a week significantly reduces heart rate, lowers BMI and decrease HDL and LDL levels thus, enhancing performance levels of individuals as compared to (45-55 minutes) exercise performed at the rate of 2-3 times a week (Noe et al., 2014).

Trials on Model Organisms

Obesity has been linked to several vascular disorders in humans, mainly due to a diet consisting of high-fat content and a lack of physical activities. The chances of developing CVDs increase significantly with physical inactivity combined with a diet consisting mostly of high saturated fats. Preventative measures like exercise and low-fat containing diet, which reduces the risk factors influencing our cardiac health have been studied in model organisms.

Tests carried out on mouse models have shown a clear link between exercise and effects of a high-fat diet on cardiac conditions in Table 3, which can help in designing effective treatments against CVDs. Trials carried out on model organisms can help provide data for attaining new drug therapies, which in turn can lead to the treatment of various vascular disorders.

Model organisms are being used for functional studies of arterial systems. In vivo experiments using optical coherence

tomography (OCT) have been utilized for analysis of the vascular function of arteries in mouse models. The OCT method allows for determining flow resistance and inner diameter changes in the arteries of model organisms. Techniques like OCT can help us in detecting early-stage vascular dysfunction in mouse strain. thus, providing a better understanding of the disease mechanism (Muller et al., 2017).

CONCLUSION AND FUTURE PROSPECTS

Looking forward to the future of drug development and strategies for contending against the increased rate of CVDs, it has become relatively clear that newly developed treatment might not only be as economically sufficient for every individual suffering from a vascular disorder. The goal for health care providers and researchers should be to develop low-fat containing diet plans, in addition to physical training programs for patients suffering from CVDs.

Public health measures against CVDs involve costly medication, which also has adverse side effects on individual health. The increased cost and side effects of modern medication have raised widespread concerns regarding the treatment strategies not only in developing countries but also in developed countries as well. The best way to combats CVDs is to employ preventive measures before the health outcome emerges and become injurious to a person's health. Although there has been an increase in the rate of CVDs across the world, combining medicine and preventative measures with a healthy lifestyle can help reduce the risk of

developing CVDs. it is a common public expression that "prevention is better than cure". Combining physical activities with the consumption of low-fat containing a nutritional diet is the first step towards managing risk factors and preventing CVDs**.**

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COMPLIANCE WITH ETHICAL STANDARDS

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Table 3: Consequence of High fat diet (HFD) and Exercise on various mouse model.

Experiments	Results	References
Active Mouse; **(fed with HFD; 16 weeks/ provided exercise on running wheel)** Inactive Mouse; **(fed with HFD; 16 weeks/ provided NO exercise)**	In the case of the active mouse; exercise prevented diastolic dysfunction; HO-1 protein level increased. In the case of the inactive mouse; Increased cardiac stress is observed with an increased risk of diastolic dysfunction.	(Bostick et al., 2017)
Experimental Mice; **(fed with HFD; 20 weeks/ provided moderate and high-intensity exercise training)** Controlled Mice; **(fed with HFD; 20 weeks/ NO exercise provided)**	In experimental mice; Exercise prevented cardiac dysfunction; Increased aerobic activity and decreased insulin resistance. In controlled mice; No change in aerobic activity; No decrease in obesity.	(Boardman et al., 2017)
Obese Mice Group; **(fed with HFD; Provided training exercise)** Obese Mice Group; **(fed with HFD; NO training exercise provided)**	Exercise decreases intramuscular triglyceride levels by activating lipolysis factors; thus, playing an important role in lowering obesity in the active mice as compared to in the Inactive mice.	(Ko et al., 2018)
Obese Mice (Group A); **(fed with high fat and sugar water diet; Provided high-intensity exercises with intermediate fasting)** Obese Mice (Group B); **(fed with high fat and sugar water diet; NO intervention)**	Exercise with intermediate fasting helps lower weight gain, lowers LDL levels and prevents fat accumulation in mice group provided with exercise as compared to in mice group with no intervention despite taking High-fat diet.	(Wilson et al., 2018)
Trained Obese Mice; **(fed with HFD; 16 weeks/ provided physical activity)** Untrained Obese Mice; **(fed with HFD;16 weeks/ NO physical activity provided)**	Decreased level of CLK2 protein prevented fat accumulation in the liver of obese mice provided with exercise as compared to in the untrained mice group.	(Muñoz et al., 2018)

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