Abstract: The aim of current study is the risk assessment of biochemical variables of medical importance, vitamin D and calcium, in pathogenesis of Diabetes Mellitus Type II (T2DM) human beings and the risk assessment due to variation of these biochemical variables in diabetic patients. One hundred subjects (n=100) were recruited from different hospitals in Lahore (31.5204° N, 74.3587° E) after taken informed consents individually. This study was approved by the ethical committee of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore. Subjects were equally divide in two groups; diabetic (case group; n=50) and non-diabetic (control; n=50). Calcium and vitamin D levels were measured by the biochemical laboratory of Hospitals. Finally the data was statistically analyzed by using SPSS software (Version 16). A total of 52 % males and 48 % females were investigated for changes in serum calcium and vitamin D in relation to their biochemical importance in Diabetes Mellitus. Both calcium and vitamin D levels were significantly low in diabetic group (p=0.003 and p=0.001 respectively) as compare to control group. Persons correlation showed calcium and vitamin D were directly proportional to each other ($R^2= 0.577$). Odds ratio showed that the low vitamin D and low Calcium levels were 3.47 and 3.17 time more prevalent in diabetic group as compare to the non-diabetic group. Diabetes Mellitus is an increasing health issue in world causing severe morbidity and mortality. So it is important to investigate the non-conventional risk factors of Diabetes Mellitus. Thus, the current study illuminates the risk assessment of altered levels of vitamin D and calcium, their possible pathogenesis in Diabetes Mellitus. It can be concluded from this study that supplementation of vitamin D and calcium till to the normal physiological limits can help not only lowering the risk of diabetes but may also prevent its progression.

Keywords: Calcium, Vitamin D, Diabetes Mellitus, Risk Factor

Introduction
Diabetes Mellitus is a metabolic disorder that can be treated but still cannot be cured. In Pakistan there were 7.5 million cases of Diabetes Mellitus with estimated prevalence rate of 6.9% in 2017 (Al-Qahtani, 2020; Bukhsh et al., 2019). In past few years the number of diabetic patients increased exponentially and it is considered one of the leading devastating factor for the health system of our country. To cope with this everyday increasing threat, not only we have to improve the health facilities but also investigate the different contributing factors in our population. There are two major types of Diabetes Mellitus. Type I (T1DM) is also known as insulin deficient diabetes, because insulin secreting β - cells of pancreas are incapable of secreting enough amount of this hormone necessary to maintain the levels of glucose within normal physiological limits (90-180mg/dl). The other most common type is diabetes II (T2DM), also named insulin resistant diabetes. Diabetes Mellitus II is more prevalent in middle aged population and in this type receptors (GLUT) are not responding to insulin signals (Arnold et al., 2018; Umeda et al., 2020). The major well understood contributing factors in the development of Diabetes Mellitus are physical inactivity, sedentary lifestyle, obesity, smoking, excessive alcohol intake and genetic predisposition (Hruby et al., 2016; Zheng et al., 2017). Due to mild symptoms appear at the initial stages, delayed in the diagnose may lead to long term complications such as cardiovascular problems, neuropathy, retinopathy, nephropathy, muscular dystrophy, erectile dysfunctions and also a confounding factor for many malignancies like prostate and breast cancer (Kahanovitz et al., 2017; Marjanac et al., 2019). The radically high incidence rate of diabetes compels the scientists to look deep into its pathophysiology. Resent investigations showed that calcium and vitamin D are also involved in development of diabetes. Normal calcium levels have a pivotal role in the secretion of insulin. Similarly vitamin D receptors, located on the β cells...
of pancreas, aid the insulin release when serum vitamin D levels are present in normal physiological limits (Bendik et al., 2014; Van Belle et al., 2013).

Material and methods
Total of one hundred subjects (n=100) included in this case and control study. Gender stratification calculated as 52% male and 48% female. Subjected divided into two groups, case and control. All diseased (diabetics) were placed in Case group, whereas sex and age matched subjects which had no disease (non-diabetics) in control group. All confounding variables like hypertension, cardiovascular diseases, renal dysfunction, liver cirrhosis or other endocrine disorders excluded from the study. The current study was approved by the ethical committee of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore. All subjects were asked for informed consent before including them in the study. Biochemical analysis was done by the attached laboratory of hospital. Data was computed by using SPSS. 16 software. Odds ratio were calculated by using mathematical formula.

Results
The results of the current study revealed statistically significant low levels of vitamin D and calcium levels in diabetic patients as compare to the non-diabetics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-Diabetes n=50</th>
<th>Diabetes n=50</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.95±0.9</td>
<td>7.71±0.656</td>
<td>0.003</td>
</tr>
<tr>
<td>Vitamin D (ng/ml)</td>
<td>40.63±6.5</td>
<td>17.72±3.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Graph 1: comparison of Calcium levels

Graph 1 Showed that that calcium levels are significantly low in diabetic group as compare to the non-diabetes patients (p= 0.003)

Graph 2: Comparison of vitamin D levels between the groups

Graph 2 shows that the vitamin D levels in patients with diabetes are significantly low in diabetes as compare to the non-diabetics (p= 0.001)

Graph 3: Correlation between vitamin D and Calcium levels (Scatter Plot)

Table 2: Pearson’s Correlation in between Vitamin D and Calcium

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.557**</td>
</tr>
</tbody>
</table>

Pearson’s correlation showed value of 0.557 that means a moderately strong correlation between vitamin D and calcium levels. It can be interpreted as the levels of vitamin D increases the levels of calcium also increase. To assess the odds of vitamin

D and calcium levels in diabetes, odds ratio was calculated as

<table>
<thead>
<tr>
<th>Table 3: odds of a calcium deficient subjects in diabetic group</th>
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<tr>
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<tr>
<td>DEFICIENT CA LEVELS (YES)</td>
</tr>
<tr>
<td>DEFICIENT CA LEVELS (NO)</td>
</tr>
<tr>
<td>TOTAL</td>
</tr>
</tbody>
</table>

Odds ratio = \( \frac{ad}{bc} = 31x33/17x19 \)

=1023/323 =3.17

The result showed there were 3.17 times more calcium deficient subjects present in diabetic group as compare to the non-diabetic group.

<table>
<thead>
<tr>
<th>Table 4: Odds of a vitamin d deficient subjects in diabetic group</th>
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<tbody>
<tr>
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<tr>
<td></td>
</tr>
<tr>
<td>DEFICIENT VITAMIN D LEVELS (YES)</td>
</tr>
<tr>
<td>DEFICIENT VITAMIN D LEVELS (NO)</td>
</tr>
<tr>
<td>TOTAL</td>
</tr>
</tbody>
</table>

Odds ratio = \( \frac{ad}{bc} = 34x31/19x16 \)

=1054/304 =3.47

Result showed that odds of the low vitamin D in diabetic group is 3.47 times more than non-diabetic group.

**Discussion**

Diabetes Mellitus is commonly associated with a metabolic disorder that causes a higher or lesser degree of disability in the metabolism of carbohydrates, lipids, or protein. It is a multifactorial disease that may result due to some modifiable or non-modifiable factors. Sedentary life style, food habits, alcohol intake and obesity are the modifiable factors. Other non-modifiable factors are family history, gender, autoimmune disorders, and genetic predisposition. Advancement in scientific knowledge and methodology has opened a new horizon for better understanding of the role of different biochemical variable in the pathogenesis of diabetes. Recent investigation reported colossal role of vitamin D and calcium levels as a risk factor in development and progression of Diabetes Mellitus (Malik et al., 2018; Rodrigues et al., 2019). There are two sources of vitamin D in the human body, first is photo-biosynthesis and the other one is by intake of vitamin D rich foods. Active form of vitamin D is produced by the two successive hydroxylations of pre-vitamin D, first in the liver (25-hydroxylases) and then in the kidneys (1α-hydroxylase) (Khan et al., 2017; Masri et al., 2015). Active form of vitamin D can interact to different cells having vitamin D receptors (VDR). These receptors are also present on the beta cells of pancreas. The mechanism of action of vitamin D on VDR starts with the internalization of Vitamin D and VDR in plasma and subsequent heterodimerisation with the retinoid X receptor (RXR). The RXR-VDR complex bind to vitamin D response element (VDRE). After this the co-repressor (Co-R) protein from the target region is released and allow the interaction of co activator (Co-R) protein to the target region. This molecular interaction activate the RNA polymerase II transcriptional complex (POL II) and leads to the transcription of target gene (Mathieu et al., 2005). For type 2 diabetes to develop, impaired pancreatic beta-cell function, insulin resistance and systemic inflammation are often present. There are several lines of evidence to support that vitamin D influences all these pathways (Malik et al., 2020; VanAmerongen et al., 2004). A role for vitamin D in pancreatic beta-cell function might be mediated by the binding of circulating 1,25-dihydroxyvitamin D to the beta-cell vitamin D receptor. Alternatively, vitamin D could function through activation of 25-hydroxyvitamin D (25OHD) by 1- alpha-hydroxylase, which is expressed in beta cells. Vitamin D may directly enhance insulin sensitivity by stimulating the expression of insulin receptors and/or by activating peroxisome proliferatoractivated

receptor (PPAR-), a transcription factor implicated in the regulation of fatty acid metabolism in skeletal muscle and adipose tissue. Vitamin D may also affect insulin secretion and sensitivity indirectly via its role in regulating extracellular calcium concentration and flux through cell membranes in the beta cell and peripheral insulin-target tissues. Finally, the well-recognized immunomodulatory properties of vitamin D in relation to T-cell activity (Malik et al., 2020) would influence a number of steps in the autoimmune process leading to type 1 diabetes. Literature supports the above pathology showing the direct relationship of vitamin D with the Diabetes (Mitri et al., 2011; VanAmerongen et al., 2004). The results of current study reveal the manipulator effect of vitamin D in diabetes. But few studies and meta-analysis revealed no such relationship (Norris et al., 2018).

Conclusion
From the results of our current study, it can be concluded that deficiency of vitamin D leads to decreased levels of calcium which might be the contributory factors in onset of diabetes mellitus II.

Limitations
Sample size is small so further analysis with large sample size is required to reach a final conclusion.

Conflict of interest
Authors declare no conflict of interest in the current study.

Reference


D levels, but not VDR polymorphisms, influence type 2 diabetes mellitus in Brazilian population independently of obesity. *Medicina* **55**, 188.


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