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A Comparison of Somatic Symptoms between Depressive Disorder and Somatoform Disorder

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Abstract:- The prevalence of painful symptoms in depressed patients is around 65% while for somatoform disorder, it varies from 2-40% due to different study designs used across the population. Due to overlap in the diagnosis, a hypothesis has been laid that depressive disorder and somatoform disorder might be sharing a common pathway for symptom development. This study was conducted to compare the somatic symptoms between depressive disorder and somatoform disorder. 60 cases each of depressive disorder and somatoform disorder were enrolled in this study. The sample was selected on the basis of inclusion and exclusion criteria and alternate sampling method was used. HAM-D and 4DSQ scales were applied on all the cases. The qualitative data were depicted in terms of percentages and the quantitative data were expressed in terms of Mean ± SD. The statistics were done using IBM SPSS 20V. Somatoform disorder patients showed a significantly higher stress levels than patients of depressive disorder. Also, 11 items namely dizziness or feeling light headed, fainting, back pain, excessive sweating, palpitations, a bloated feeling in the abdomen, blurred vision or spots in front of your eyes, shortness of breath, nausea or an upset stomach, pain in the abdomen or stomach area and pain in the chest showed a significant trend towards somatoform disorder when compared to depressive disorder group. Future studies should investigate the various etiological factors that are predictive of symptom dimensions in depressive disorder and somatoform disorder patients.

Keywords:- *Depressive Disorder, Somatoform Disorder, 4DSQ, Pain, Somatic Symptoms, Physical Complains.*

I. INTRODUCTION

Depression and pain are commonly seen together as co morbidities. Various studies concluded a mean prevalence of moderate depressive disorder in cases associated with chronic pain as 52% and prevalence of painful symptoms in depressed patients as 65%. In one example of a primary care setting, 69.1% cases with moderate depressive disorder showed moderate levels of pain symptoms while around 38.6% without moderate depressive disorder reported moderate pain symptoms. This co-morbidity of somatic complains and depression has a very negative impact on the following aspects of a person's life:

- Higher healthcare related costs.
- More days of abstinence from work causing decrease in productivity.
- Overall chances of remission of depressive symptoms are decreased.

Patients who have any kind of residual symptoms are more likely to have a relapse of his or her depressive episode and that also before time as compared to cases without any residual symptoms (Robinson et al., 2009). Wernicke talked a lot about the term "vital feelings" to define some somatic symptoms noted in mood disorders. These included persistent exhaustibility and abnormal sensations in the whole body affecting the chest, head and the abdomen. In a study conducted in US on 573 patients with a diagnosis of moderate depressive disorder, around 69% of the cases had complains of generalised body aches. Ohayon and Schatzberg conducted a population based study and found that patients diagnosed as cases of depression with chronic somatic symptoms reported more period of depressive mood (average 19 months) than cases without any somatic symptoms (average 13.3 months). Fishbrain concluded in his study that chronic painful symptoms are a big risk factor for suicidal ideas in depression. Also, the prognosis is poor when there is pain related abstinence from work, poor state of overall health, higher use of opiates, increasing poly-pharmacy and multiple visits to the doctor for persistent somatic complains (Kapfhammer, 2006).

It has been estimated that depression has affected more than 300 million people all over the globe and adds to 7.5% of all years lived with disability in the global burden of the disease. 30-40% of cases have a recurrent episode within a year (Shinohara et al., 2019). Epidemiological studies concluded prevalence of moderate depressive disorder in patients with chronic pain symptoms is 65%. Chronic pain symptoms in depression often showcase signs of learned

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helplessness. This is the phenomenon when the person feels that the condition is not in his control and so none of the attempts are going to change the current situation. In moderate depressive disorder, the core features are low mood and loss of interest in most of the daily activities. There could also be accompanying somatic symptoms like feeling low in energy, general sensations of pain, poor concentration, impaired memory and finding it difficult to make decision. In a study, it was found that physical symptoms were reported by more than 40% of patients who responded to and were continuing antidepressive medications (Christensen et al., 2018). Depression is known to run both a chronic and recurrent course with nearly 40% of cases experiencing recurrent episodes within a year. Between the years 1990 to 2010 period, years of life lived with disability increased by 37.5% with respect to the depressive disorders (Shinohara et al., 2019). In moderate depressive disorder, symptoms should persist for most of the day, almost every day for at least two consecutive weeks. This episode must go along with social and occupational impairment also. The mood disturbed in such cases is often sad, hopeless or "down in the dumps (Lieberman & Massey, 2009) .

The common factors that predispose to medically unexplained symptoms include heredity, history of any chronic illness, early life diversity, presence of chronic distress or any type of mental illness. Along with these, various predisposing factors also play a role in the presentation of somatic symptoms like the presence of a biological stressor, any acute illness or psychiatric disorder. To add more to the problem, perpetuating factors can also add on to poor prognosis on a case to case basis like detrimental illness beliefs, misinformation, various factors at workplace, poor health related habits and poor unification with the treatment system (Richardson & Engel, 2004).

In an observational study conducted on 145 patients based on the psychical symptoms in major depressive episode, the authors confirmed that among Puerto Rican population, somatic symptoms were quite common when diagnosed as depression. They observed a consistent relationship between depression and pain with $p< 0.0001$. It was also found that all these physical symptoms were not reported to the psychiatrists which led to a major impact on the benefits the antidepressants could provide for the patients (Tamayo et al., 2005). Mia et al., 2005 conducted a study for the assessment of somatic symptoms, primarily gastrointestinal complains that were unexplained by any medical cause in patients of depression. They studied 1165 patients and the study revealed that complains regarding gastrointestinal tract system are very common in depressed patients. Most common complain noted was decreased appetite (67.7%) that was more in females than males followed by constipation (57.7%). They concluded that patients seeking help followed more of various types of treatment rather than consulting a psychiatrist as there is less awareness for the same (Afridi et al., 2005).

ICD-10 defines somatization as repeatedly presenting somatic symptoms along with persistent requests for a lot of investigations irrespective of repeated negative reports and reassurances by the respective doctor that the physical symptoms do not have any physical basis. Somatoform patients often undergo inadequate diagnosis and are often exposed to a lot of investigations. The prevalence of somatic pain varies from 2-40% due to different study designs used across the population. Medically unexplained physical symptoms comprise of mostly female gender belonging to the middle age group with gastrointestinal symptoms being the commonest out of all complains. Out of the gastrointestinal symptoms, dysmotility is the most common symptom in 23% of men and 27% of women (Smith, 1992). Due to overlap in the diagnosis, a hypothesis has been laid that depressive disorder and somatoform disorder might be sharing a common pathway for symptom development (Rief et al., 2010).

➢ *Aims and Objectives*

This study was conducted to compare the somatic symptoms between depressive disorder and somatoform disorder.

II. MATERIALS AND METHODS

This study design was reviewed by an appropriate ethical committee. An informed consent of the participants was obtained after the nature of the procedures had been fully explained.

STUDY DESIGN

The present study was a hospital based observational study.

SAMPLE

The study was done on 60 depressive disorder patients (as per ICD 10 Clinical descriptions and diagnostic guidelines and individual HAM-D score greater than 14) and 60 somatoform disorder patients (as per ICD 10 Clinical descriptions and diagnostic guidelines and individual HAM-D score less than 14) .The sample was selected on the basis of inclusion and exclusion criteria and alternate sampling method was used for selection of patients from Outpatient and Inpatient departments of Psychiatry.

INCLUSION CRITERIA

- Patients fulfilling the ICD-10 DCR (1993) criteria for depressive disorder and somatoform disorder.
- Both male and female patients between ages 18-45 years will be included.
- Those giving informed written consent for this study.
- No history of organicity like epilepsy, stroke or significant head injury.

EXCLUSION CRITERIA

- Those not giving informed consent.
- Suffering from co-morbid medical/neurological illness
- Substance dependence except nicotine/caffeine.

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TOOLS

1. Sociodemographic data

This will be specially prepared for noting down the social, demographic & clinical variables of the patient including Case Record File number, age, sex, education level, occupation, marital status, religion, family income, duration of illness, number of hospitalizations, past history, family history and diagnosis of patient (ICD-10 DCR).

2. The Hamilton Rating Scale for Depression

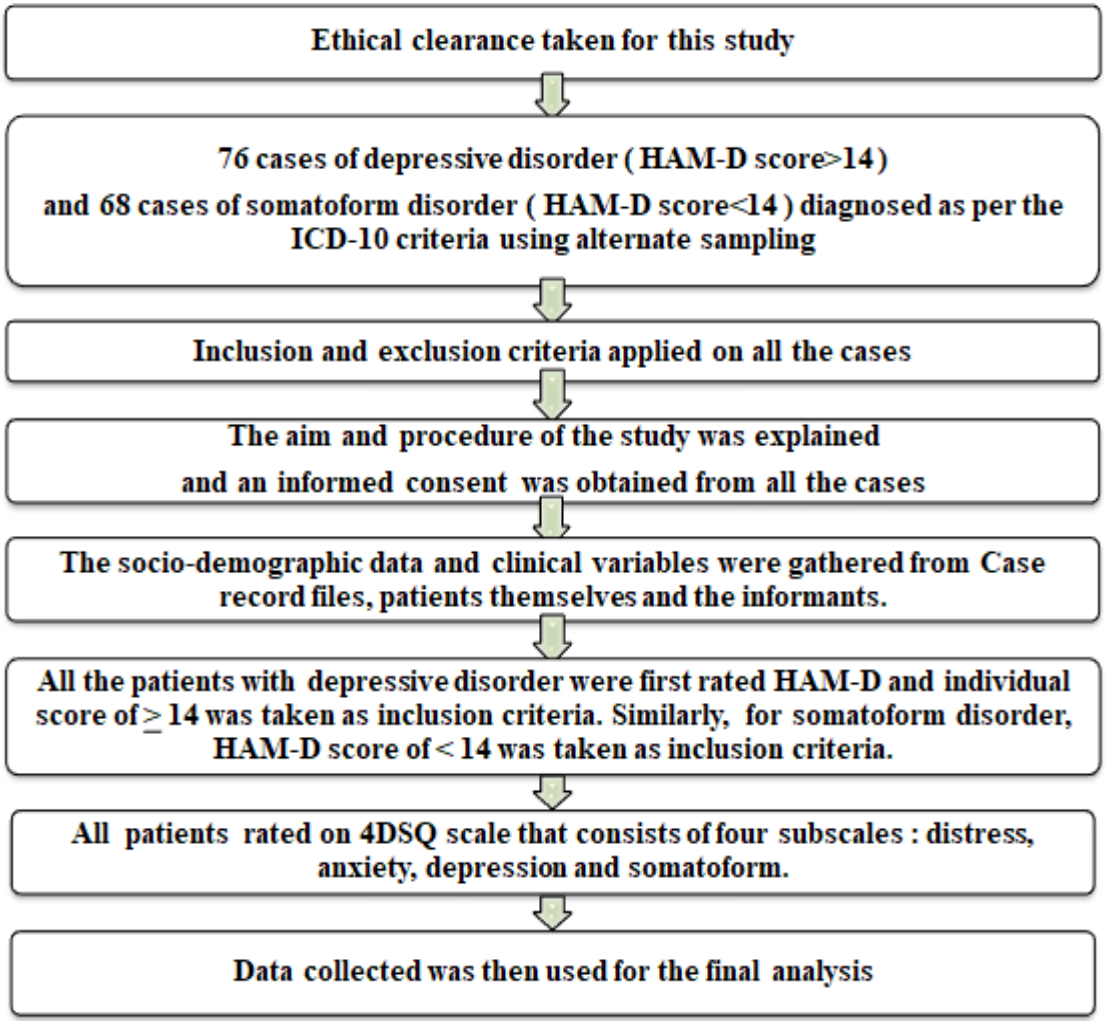
HRSD/HAM-D is a 17-21 items observer rated scale to assess presence and severity of depressive state in patients. In this scale, 9 items are scored 0-4, while further 8 are scored 0-2, as these represent variables which don not lend themselves to quantitative rating (0= absent; 1= doubtful or slight; 2= mild; 3= moderate; 4= severe. 0= absent; 1= doubtful or slight ; 2= clearly present). Items 18-21 are not regarded as measuring the intensity of depression and commonly omitted. A score of 11 is generally regarded as indicative of depression. This tool has been widely evaluated and established as a highly reliable and valid assessment tool.

3. The Four Dimensional Symptom Questionnaire (4DSQ)

It consists of a list of questions about various complaints and symptoms that the patient may have. Each question refers to the complaints and symptoms that the patient had in the past week (the past 7 days, including the present day). It works on four aspects of psychological symptoms: anxiety, depression, distress and somatization. It helps to formulate the diagnosis and treatment plan of the patient. The above listed four dimensions of the 4DSQ scale enclose almost the whole range of psychosomatic and psychological complains. It has a total of 50 items encompassing the concerned complains in the past one week (Terluin et al., 2008). Scoring of 4DSQ scale: The scale has each item available for 0, 1 or 2 points scoring where:

- 0 points: symptoms are absent.
- 1 point: symptoms 'sometimes' present.
- 2 points: regularly or more often present.

III. PROCEDURE



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IV. STATISTICAL ANALYSIS

It was done with the help of the Statistical Package for Social Sciences-20 (SPSS-20). In both the groups, the socio-demographic variables (both continuous and discrete data) 'were summarized with using frequency, percentages, mean and standard deviation as per the applicability. For measuring the difference amongst various socio-demographic and clinical variables, chi square test was applied for discrete variables. For continuous variables, T-test was applied. Independent T test was used to examine the difference between depressive disorder and somatoform disorder groups.

V. RESULTS

Table 1: The socio-demographic characteristics of Depressive disorder and Somatoform disorder

S. No.	Variables		Depressive disorder (N=60) *n (%)*	Somatoform disorder (N=60) *n (%)*	$\chi 2$	df	p
1	Gender	Male	29 (48.3%)	10 (16.7%)	13.71	1	.000**
		Female	31 (51.7%)	50 (83.3%)			
2	Domicile	Urban	24 (40%)	23 (38.3%)	0.035	1	.899
		Rural	36 (60%)	37 (61.7%)			
3	Occupation	Employed	41(68.3%)	26 (43.3%)	7.60	1	.005**
		Unemployed (student,HW)	19 (31.7%)	34 (56.7%)			
4	Marital status	Married	52 (86.7%)	58 (96.7%)	3.93	1	.047*
		Single	8 (13.3%)	2 (3.3%)			
5	Family type	Nuclear	48 (80%)	49 (81.7%)	0.054	1	.500
		Joint	12 (20%)	11 (18.3%)			
6	Family income (In Rs.)	<10,000	37 (61.7%)	43 (71.7%)	1.35	1	.166
		>10,000	23 (38.3%)	17 (28.3%)			
7	Family history of psychiatric illness	Present	14 (23.3%)	10 (16.7%)	0.000	1	.596
		Absent	46 (76.6%)	50 (83.3%)			
8	Age (in years)	18-30	25 (41.7%)	3 (5%)	22.55	1	.000**
		31-45	35 (58.3%)	57(95%)			

* Correlation is significant at 0.05 level
** Correlation is significant at 0.01 level

Table 1 shows the comparison of socio-demographic variables (the categorical one as well as continuous variable that is the age) between the two groups (depressive disorder and somatoform disorder). There'were'29 (48.3%) males and 31 (51.7%) females in depression group whereas 10 (16.7%) males and 50 (83.3%) females were there in the somatoform group (χ^2 13.71; p= .000). There were 25 (41.7%) aged 18-30 years and 35(58.3%) aged 31-45years in depressive disorder group whereas in somatoform disorder, there were 3(5%) in the age group 18-30 years and 57(95%) in the age group of 31-45 years (χ^2 =22.55; p= .000). Among the depression group 68.3% were employed as compared to employment of 43.3% among somatoform group. There were significant differences with respect to occupational status (χ^2=7.60; p=0.05) between the two groups. The two groups were also comparable with respect to marital status (p=.047), family type (p=.500), economic status (p=.166) and family history of any psychiatric illness (p=.596). Among depressive disorder, 86.7% were married and 13.3% were unmarried; 80% were from nuclear family and 20% were from joint family; 23.3% had family history of any psychiatric illness and 76.6% did not have any family history of psychiatric illness. Among somatoform group, 96.7% were married and 3.3% were unmarried; 81.7% were from nuclear family and 18.3% were from joint family; 16.7% had a positive family history of psychiatric illness while 83.3%'did not have any family history of psychiatric illness.

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Table 2: The baseline scores of 4DSQ scale on Day 1 with reference to the socio-demographic characteristics

S. No.	Variables		4-DSQ Mean ± SD (N=120)	df	p
1.	Age (in years)	18-30	50.43±8.48	118	.512
		31-45	56.15±9.84		
2.	Gender	Male	52.62±11.19	118	.050*
		Female	55.88±8.96		
3.	Domicile	Urban	54.94±10.54	118	.344
		Rural	54.74±9.40		
4.	Occupation	Employed	54.27±9.93	118	.764
		Unemployed (student, HW)	55.51±9.72		
5.	Marital status	Married	54.87±10.15	118	.092
		Single	54.20±5.20		
6.	Family type	Nuclear	54.65±9.48	118	.347
		Joint	55.52±11.33		
7.	Family income (In Rs.)	<10,000	52.90±9.67	118	.695
		>10,000	55.78±9.81		
8.	Family history of psychiatric illness	Present	55.80±11.17	118	.377
		Absent	54.62±9.58		

*Correlation is significant at 0.05 level

Table 2 shows the baseline scores 4DSQ scale on day 1. The mean score for age group of 18-30 years was 50.43±8.48 (SD) and 56.15±9.84 (SD) for the age group of 31-45 years with no statistical significance noted between the two age groups (p=.512). For the gender comparison, mean was found to be 52.62±11.19 (SD) for the males and 55.88±8.96 (SD) for female group, showing a significant p value of .050 which was statistically significant showing a trend towards the male group. The mean for urban domicile group was 54.94±10.54 (SD) and 54.74±9.40 (SD) for the rural group and showed no statistical significance (p=.344). The employed group had a mean of 54.27±9.93 (SD) and the unemployed group had a mean of 55.51±9.72 (SD) which was not significantly significant (p=.764). The mean for married group was 54.87±10.15 (SD) and 54.20±5.20 (SD) for the unmarried group which did not come out to be significantly significant (p= .092). Nuclear family group had a mean score of 54.65±9.48 (SD) and the joint family group had a mean of 55.52±11.33 (SD) which and was not statistically significant (p=.347). The economic status group with an average income of more than Rs.10,000 was 55.78±9.81 (SD) and the mean for average income of less than Rs.10,000 was 52.90±9.67 (SD) that was not significant statistically (p=.695). The group that had a positive family history of some psychiatric illness had a mean score of 55.80±11.17 (SD) and the group with no family history of any psychiatric illness had a mean of 54.62±9.58 (SD) that were not statistically significant (p=.377).

Table 3: Comparison of 4DSQ anxiety and distress item subscale between depressive disorder and somatoform disorder group

S. No.	Variable	Group	Mean ± SD	df	t	p
1.	total 4DSQ-anxiety subscale	Depressive disorder	12.20 ±4.86	58	.167	.906
		Somatoform disorder	12.35 ±4.96			
2.	total 4DSQ-distress subscale	Depressive disorder	13.20 ±3.41	58	14.41	.000**
		Somatoform disorder	23.52 ±4.37			

**Correlation is significant at 0.01 level

Table 3 shows the other 2 subscales of 4DSQ scale that were anxiety and distress. The comparison was done between depressive group and somatoform group and showed that somatoform group had higher levels of distress as compared to the depressive group that was statistically significant (p=.000). It did not reach a statistical significance in terms of anxiety (p=.906).

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Table 4: Comparison of 16 items of somatoform subscale of 4DSQ scale between depressive disorder and somatoform disorder

S. No.	During the past week, did you suffer from:	Diagnosis	Mean ± SD (N=60)	p
1.	Dizziness or feeling light headed	Depressive disorder	.87±.536	.001**
		somatoform disorder	1.52±.651	
2.	Painful muscles	Depressive disorder	1.22±.640	.088
		somatoform disorder	1.68±.469	
3.	Fainting	Depressive disorder	.03±.181	.000**
		somatoform disorder	.92±.889	
4.	Neck pain	Depressive disorder	1.00±.611	.107
		somatoform disorder	1.63±.520	
5.	Back pain	Depressive disorder	.10±.303	.000**
		somatoform disorder	1.48±.567	
6.	Excessive sweating	Depressive disorder	.03±.181	.000**
		somatoform disorder	.38±.666	
7.	Palpitations	Depressive disorder	.15±.404	.000**
		somatoform disorder	.67±.837	
8.	Headache	Depressive disorder	1.23±.621	.549
		somatoform disorder	1.25±.571	
9.	A bloated feeling in the abdomen	Depressive disorder	.00±.000	.000**
		somatoform disorder	1.40±.616	
10.	Blurred vision or spots in front of your eyes	Depressive disorder	.03±.181	.000**
		somatoform disorder	.48±.792	
11.	Shortness of breath	Depressive disorder	.07±.252	.000**
		somatoform disorder	.92±.869	
12.	Nausea or an upset stomach	Depressive disorder	.00±.000	.000**
		somatoform disorder	.87±.769	
13.	Pain in the abdomen or stomach area	Depressive disorder	.28±.524	.000**
		somatoform disorder	.57±.810	
14.	Tingling in the fingers	Depressive disorder	1.05±.746	.444
		somatoform disorder	1.27±.607	
15.	Pressure or a tight feeling in the chest	Depressive disorder	.22±.454	.178
		somatoform disorder	.95±.649	
16.	Pain in the chest	Depressive disorder	.15±.444	.000**
		somatoform disorder	.65±.709	

**Correlation is significant at 0.01 level

Table 4 summarizes the scores of 16 items given under the heading of somatoform in the 4DSQ scale. The comparison of these scores was done for day 1 of all the cases. It was found that on day 1, there were significant findings statistically in 11 items that were dizziness or feeling light-headed (p=.001), fainting (p=.000) , back pain (p=.000), excessive sweating (p=.000), palpitations (p=.000), a bloated feeling in abdomen (p=.000), blurred vision or spots in front of the eyes (p=.000), shortness of breath" (p=.000), nausea or an upset stomach (p=.000),pain in abdomen or stomach area (p=.000),and pain in the chest (p=.000). All these 11 items showed a significant trend towards somatoform disorder. It did not reach statistical significance in terms of painful muscles (p=.088), neck pain (p=.107), headache (p=.549), tingling in fingers (p=.444) and pressure or a tight feeling in the chest (p=.178) for day 1 of 4DSQ score of the 16 sub items.

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VI. DISCUSSION

Socio Demographic and Clinical Characteristics

Demographic characteristics of the two patient groups have been shown in table 1 and clinical characteristics have been shown in table 2. Comparison of the socio-demographic and clinical profile between depressive and somatoform groups showed that the two groups were comparable in domicile, family type, family income and any family history of psychiatric illness. But, they were not comparable in terms of age and sex (p=≤.001) distribution and in occupation (p=0.005) and the marital status (p=0.047). The somatoform group had more of females (95%) than the males (5%). All the patients in both the groups were diagnosed as cases using ICD-10 criteria and two scales applied on day 1 and day 42 of the interview that were HAM-D scale and the 4DSQ scale. Mean age of patients in depression group was 32.58±6.92 years and 38.30±4.21 years in somatoform group which was different as compared to study by Lim & Kim, 2005 who did not have any significant difference in their age groups. One striking feature was that out of 60 patients there were only 31 female patients (51.7%) compared to 29 male patients in depression groups and only 50 female (83.3%) patients compared to 10 male patients in somatoform group. Thus, in our study both the groups had significant differences in gender, marital status and occupation between the depressive disorder and somatoform group. The studies in India show two fold greater prevalence of depressive disorder between males and females. For somatoform disorder, Indian population studies showed equal prevalence in both genders. Indu et al., 2017 conducted a cross-sectional study in six primary care settings and evaluated 827 adult outpatients diagnosed as depression using the ICD-10 Diagnostic Criteria for Research. Overall the depression prevalence was 27.2% and was found to be higher in women (9.2%) than men (3.6%). In relation to somatoform disorder, Baitha et al., 2019 conducted a study on 976 patients and found prevalence was significantly higher in females that was similar to our study. As per the works of Ramachandra et al., 2013, for general hospital and primary health care settings, the prevalence rate has been estimated to be around 0.5%. The authors also included another diminished form of somatisation characterized by three or more medically unexplained but currently bothersome symptoms along with a two year history of somatisation has a prevalence of`8.2% in 4 primary cares. All the patients in depression group were diagnosed using the ICD-10 criteria and their HAM-D score > 14 and all the somatoform patients were also diagnosed using the ICD-10 criteria.

Comparison of the anxiety and distress subscales of 4DSQ scale in depressive disorder and somatoform disorder groups on day 1

Also, comparison of 4DSQ anxiety and distress item subscale between depressive disorder and somatoform disorder group on day 1 was done and it showed that somatoform group had higher levels of distress as compared to the depressive group that was statistically significant (p=.000) while it did not reach any statistical significance in terms of anxiety (p=.906). Work done by Harris et al., 1996 to determine the prevalence of anxiety and depression in general practice patients included 4867 patients (85%) who completed questionnaires suitable for analysis. 36% per cent of these patients had abnormal scores on a General Health Questionnaire (GHQ-12) and they were more likely to be women or to be unemployed. 20% of these patients had been treated for depression or anxiety in the previous 12 months, 52% were prescribed drug therapy and were more likely to be older male or unemployed.

Comparison of somatic symptoms between depressive disorder and somatoform disorder

This study examines the issue of somatic symptoms, as evaluated by 4DSQ in depression and somatoform disorders. The 4DSQ scale applied on all cases at day 1 had a mean of 50.32±9.05 (SD) and 59.32±8.44(SD) for somatoform disorder and found to be of insignificant value (p=.635). Lieb et al., 2007 discussed about the comorbidity between somatoform disorder and depressive disorder. As per their work, the association was spurious resulting from various methodological problems like reporting or recalls bias that explained the observed comorbidity or similar physical symptoms accounting for more than one diagnosis. They also highlighted that the complex associations like somatoform disorders may not influence the onset of depressive disorders, but they may influence remission or treatment responses. Löwe et al., 2008 conducted a study on 2091 consecutive primary care clinic patients in a multicenter cross sectional survey in 15 primary care clinics. They concluded that in nearly 50% of the total cases, comorbidities existed between depression and somatization. The contributions of the commonalities of depression and somatization to functional impairment substantially exceeded the contribution of their independent parts. In our study it was found that on day 1, there was significant findings statistically in 11 items that were dizziness or feeling light-headed, fainting, back pain, excessive sweating, palpitations, a bloated feeling in the abdomen, blurred vision or spots in front of your eyes, shortness of breath, nausea or an upset stomach, pain in the abdomen or stomach area and pain in the chest (p ≤.001). All these 11 items showed a significant trend towards somatoform disorder. It did not reach statistical significance in terms of painful muscles (p=.088), neck pain (p=.107), headache (p=.549), tingling in the fingers (p=.444) and pressure or a tight feeling in the chest (p=.178) for day 1 4DSQ score of the 16 subitems. In a study conducted by Bekhuis et al., 2016 it was found that somatic symptoms are predictors of a worse prognosis of depressive disorder that is independent of psychiatric characteristics, somatic diseases, lifestyle factors, and disability. These results stressed the significance of considering somatic symptoms in the diagnosis as well as the treatment trajectory of patients with depression. We also evaluated the other 2 subscales of 4DSQ scale that were anxiety and distress. The comparison was done between depressive group and somatoform group on day 1 and showed that somatoform group had higher levels of distress as compared to the depressive group that was statistically significant (p=.000). It did not reach a statistical significance in terms of anxiety (p=.906).

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VII. CONCLUSION

On the first day of interview, somatoform disorder showed a significantly higher stress levels than patients of depressive disorder. 11 items that were dizziness or feeling light headed, fainting, back pain, excessive sweating, palpitations, a bloated feeling in the abdomen, blurred vision or spots in front of your eyes, shortness of breath, nausea or an upset stomach, pain in the abdomen or stomach area and pain in the chest showed a significant trend towards somatoform disorder when compared to depressive disorder group.

LIMITATIONS

The sample size for this study was small. Also, there was no follow-up done after the first visits of the patients. Our sample was not randomized and since it was a tertiary hospital based study, it did not represent the population at community level.

CONFLICTS OF INTEREST: There are no conflicts of interest.

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Declarations of interest: None

Ethics: This work has been approved by the appropriate ethical committees related to the institution in which it was performed and all the subjects gave informed consent to the work.

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