CHAPTER 2

REVIEW OF LITERATURE

The current study was planned to determine the efficacy of the human microbiome in influencing the mental health. Potential benefits of psychobiotics in improving mild to moderate depression status were assessed, to exhibit a connection between gut and brain axis. The present chapter also intends to open up new lines of present and imminent aspects of our investigation entitled 'Role of Fructooligosaccharide, Buttermilk and Biogenic metabolites released from fermented beverage (Ambil) as a communicator between gut and brain.'

Following flow will be followed to read the collected literature about the present research:

2.1 Incidence and prevalence of mental disorders

2.2 Incidence and prevalence of depression

- 2.2.1. The burden of depression across the states of India
- 2.2.2. Gender wise prevalence of depression

2.3 History of depression

2.4 Symptoms of depression

2.5 Depression and its types

2.6 Measuring depression

- 2.6.1 Inventory scales
- 2.6.2 Biomarkers for depression assessment

2.7 Unmasking the Invisible World: The Microbiome

- 2.7.1 Glimpse at human gut microbiota
- 2.7.2 Shaping of human gut through life span

2.8 Leaky gut leads to brain functional disorientation

2.9 Gut -Brain axis

Medium of crosstalk between microbiota–gut–brain axis

- 2.9.1 Vagus nerve
- 2.9.2 Neuroendocrine: hypothalamic–pituitary–adrenal axis
- 2.9.3 Neuropeptides
- 2.9.4 Short chain fatty acid
- 2.9.5 Neurotransmitters

. . . social and economic impact of mental disorders, including mental disabilities, is diverse and far-reaching – World Health Assembly, 2012 [WHA65/2012/REC/1]

2.1 Incidence and prevalence of mental disorders

WHO defines health as state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. However, only mortality focusing on total number of life loss is often considered as measure of health, and the effect of 'mental disorder' on an individual's wellbeing is often underestimated (Prince et al., 2007).

Mental health implies to emotional, behavioral and cognitive wellbeing which helps preserve the balance between life activities and responsibilities maintaining psychological resilience. The WHO declaration about mental health is also clear: it is "a state of well-being in which the individual realizes his or her own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her community " (World Health Organization, 2004).

For an encompassing view on health outcome of any society, both mortality and morbidity (the prevalent diseases) together known as 'burden of disease' should be taken into account. This is measured by a metric called 'Disability Adjusted Life Years '(DALYs) i.e., years lived with health burden. Conceptually, one DALY is the equivalent of losing one year of healthy life because of disease or disability (Ferrucci et al., 2007).

The disease burden has been broken down into key categories by epidemiologists into non- communicable (NCDs) communicable, maternal, neonatal and nutritional diseases and injuries (Roser and Ritchie, 2018). According to a 2017 study conducted by the Institute for Health Metrics and Evaluation (IHME), noncommunicable diseases account for 62 percent of total disease burden, communicable diseases account for 28 percent, maternal, neonatal, and nutritional diseases account for 28 percent, and injuries account for just over 10 percent, as shown in Figure 2.1. In India, 60 percent of NCDs contribute to DALYs (Figure 2.3). According to IHME data, 2016, 121.264 and 109.46 million people worldwide suffer from mental and neurological disorders, respectively (Figure 2.2). Anxiety

disorders are the leading contributors, followed by depressive disorders (Figure 2.5 and Table 2.2). The graph for India showed that 22.10 million people had mental disorders and 17.33 million had neurological disorders (Figure 2.4).

This data indicates an increase in graph peak over time, indicating an increase the number of people with mental disorders to 792 million, slightly more than one in every ten people worldwide (Global Burden of Disease Study, 2017).

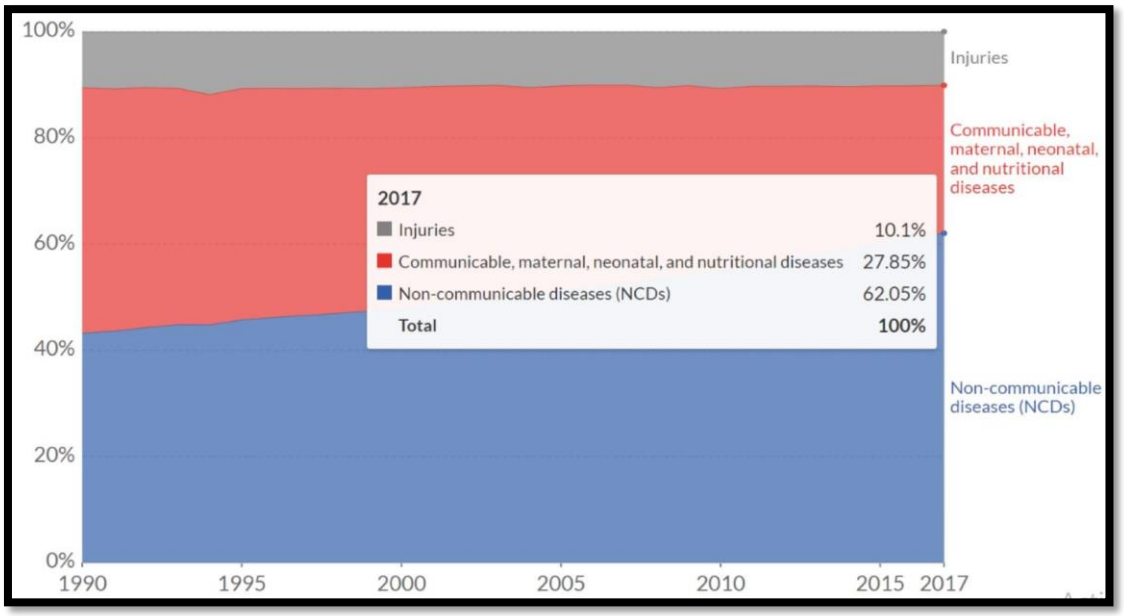

Figure 2.1: Total disease burden by cause in World.
Source: IHME, Global Burden of Disease, 2017

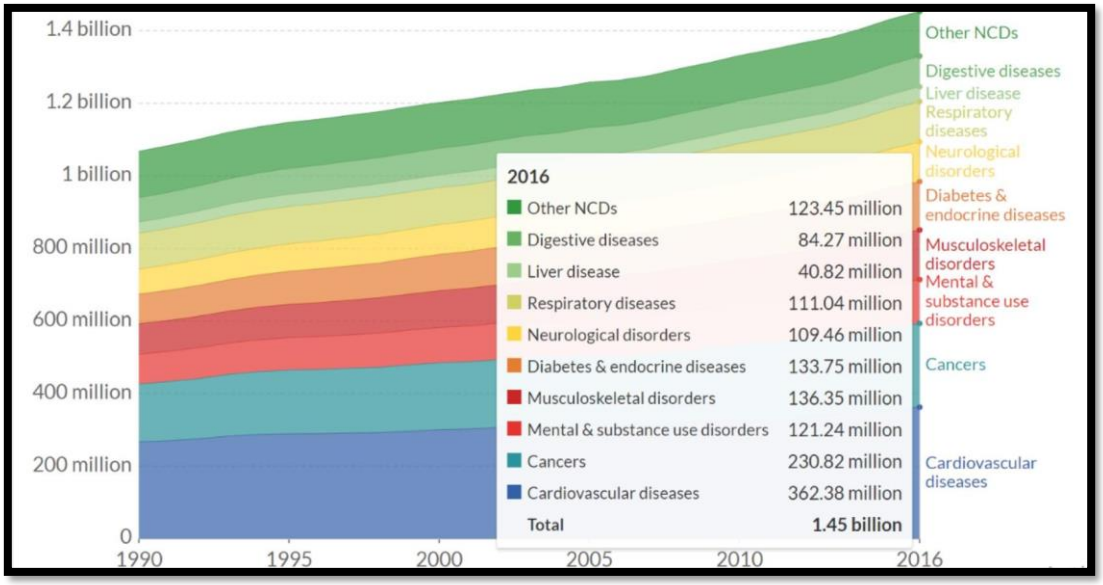

Figure 2.2: Disease burden by NCD in World.
Source: IHME, Global Burden of Disease, 2017

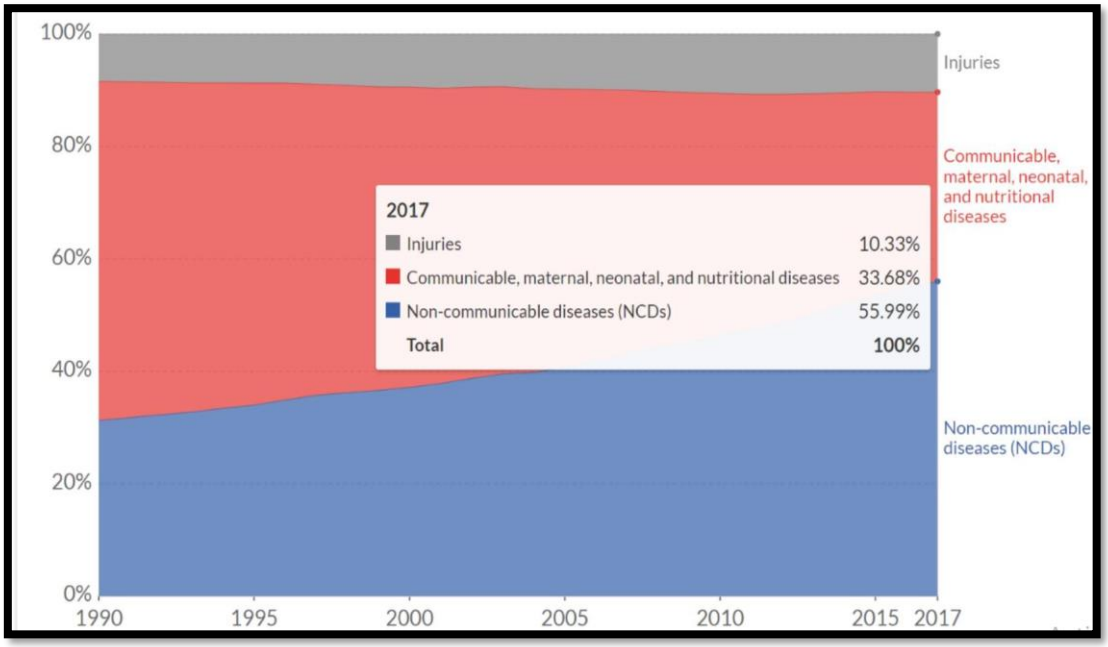

Figure 2.3: Total disease burden by cause in India.
Source: IHME, Global Burden of Disease, 2017

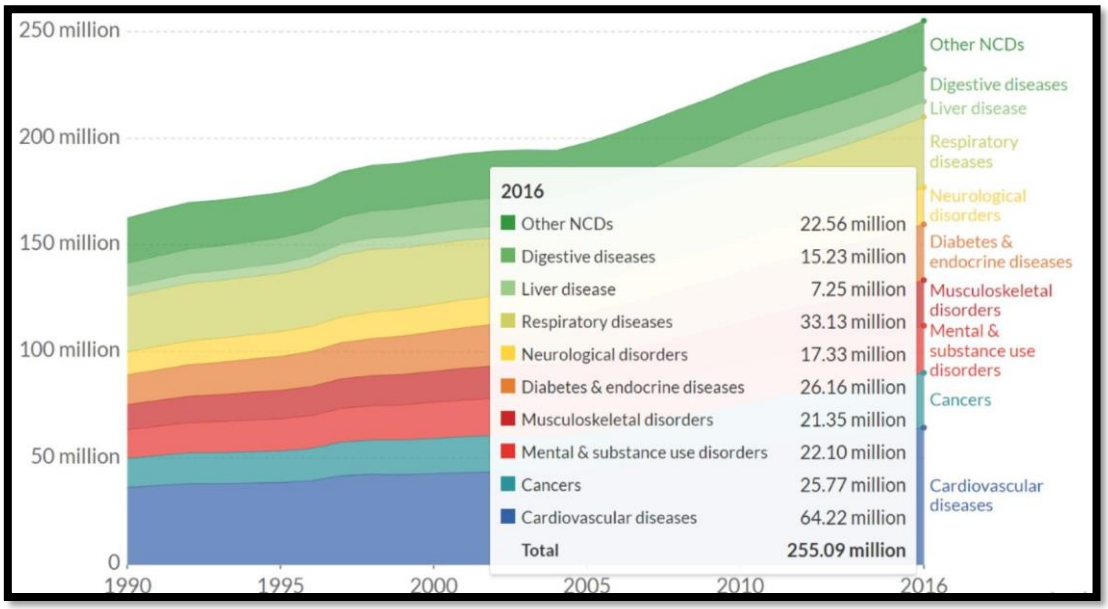

Figure 2.4: Disease burden by NCD in India.
Source: IHME, Global Burden of Disease,2017

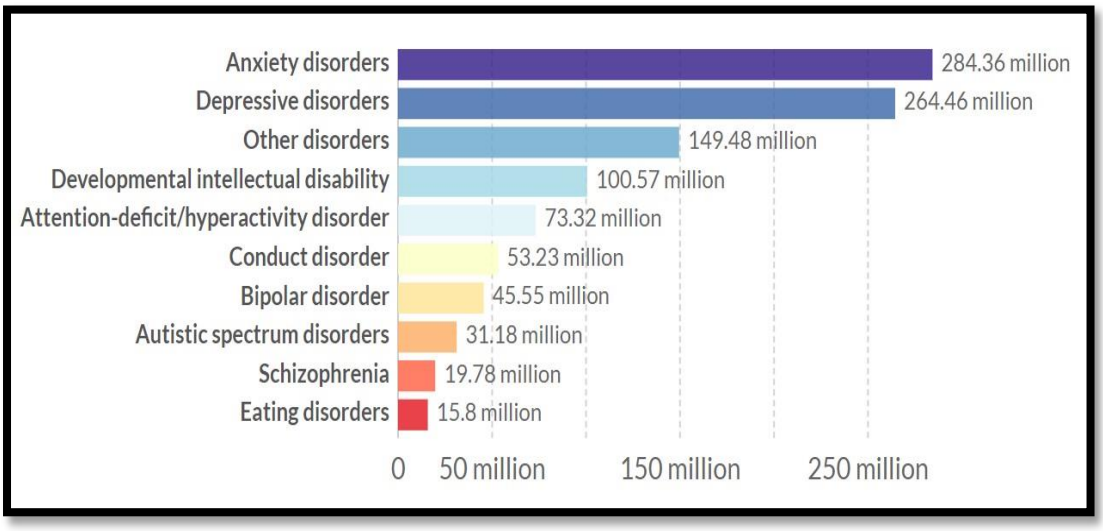

Figure 2.5: Neurodevelopmental disorder by type, World. Source: IHME, Global Burden of Disease, 2017

Table 2.1: Neurodevelopmental disorder by type, Indian states 2017: State-Level Disease Burden Initiative Mental Disorders Collaborators (Sagar et al., 2020).

Ratio of the state DALY rate to median DALY rate for all states
<0·75 | 1·00–1·24 | 1·50–1·74
0·75–0·99 | 1·25–1·49

	Crude DALY rate per 100 000 population (95% uncertainty interval)									
	Depressive disorders	Anxiety disorders	Idiopathic developmental intellectual disability	Schizophrenia	Bipolar disorder	Conduct disorder	Autism spectrum disorders	Eating disorders	Attention-deficit hyperactivity disorder	Other mental disorders
India (1380 million population)	**550 (390–748)**	**309 (220–414)**	**175 (95–283)**	**160 (121–198)**	**113 (71–165)**	**96 (58–154)**	**53 (36–73)**	**36 (23–52)**	**5·0 (3·0–8·1)**	**131 (86–181)**
Low SDI states (675 million population)	**467 (332–635)**	**294 (209–393)**	**213 (118–341)**	**143 (107–177)**	**106 (66–155)**	**108 (65–172)**	**54 (37–74)**	**31 (19–45)**	**5·1 (3·0–8·3)**	**120 (79–167)**
Bihar	406 (287–552)	292 (206–392)	252 (140–401)	133 (100–165)	102 (64–151)	117 (70–186)	54 (37–74)	26 (16–38)	5·3 (3·0–8·7)	114 (75–158)
Madhya Pradesh	471 (332–643)	268 (188–362)	207 (115–333)	147 (110–183)	106 (66–159)	101 (60–161)	53 (36–73)	32 (20–48)	5·0 (3·0–8·2)	123 (81–171)
Jharkhand	476 (337–648)	318 (224–426)	192 (104–311)	146 (108–182)	107 (67–159)	118 (72–190)	53 (36–74)	33 (20–47)	5·1 (3·0–8·4)	121 (79–168)
Uttar Pradesh	443 (316–605)	290 (204–389)	215 (118–345)	137 (102–171)	104 (64–153)	112 (66–178)	54 (37–75)	30 (19–44)	5·2 (3·1–8·5)	118 (78–164)
Rajasthan	444 (314–606)	312 (220–420)	196 (109–315)	148 (110–184)	109 (68–159)	105 (63–168)	54 (37–75)	34 (22–51)	4·9 (2·9–7·8)	122 (80–169)
Chhattisgarh	444 (312–605)	275 (194–370)	181 (98–298)	154 (115–192)	110 (69–163)	96 (57–153)	52 (36–73)	35 (22–51)	4·9 (2·9–7·8)	127 (83–175)
Odisha	720 (504–971)	316 (225–423)	186 (102–298)	163 (122–203)	112 (71–167)	89 (53–142)	52 (35–72)	34 (21–50)	4·6 (2·8–7·5)	135 (89–186)
Assam	550 (387–749)	307 (215–413)	201 (110–322)	152 (113–189)	108 (67–162)	99 (60–158)	53 (36–73)	33 (21–48)	5·0 (3·0–8·2)	127 (83–176)
Middle SDI states (387 million population)	**613 (430–828)**	**321 (228–430)**	**155 (82–251)**	**173 (129–215)**	**119 (75–175)**	**86 (51–137)**	**53 (36–72)**	**39 (25–57)**	**4·8 (2·8–7·7)**	**139 (92–192)**
Andhra Pradesh	793 (555–1065)	328 (234–436)	151 (81–246)	177 (132–219)	121 (76–177)	82 (49–132)	52 (35–71)	38 (24–56)	4·5 (2·7–7·3)	143 (94–198)
West Bengal	535 (375–720)	331 (233–444)	189 (104–300)	176 (131–219)	120 (76–179)	87 (52–139)	52 (36–72)	37 (23–54)	4·7 (2·7–7·6)	141 (93–196)
Tripura	505 (359–688)	323 (227–437)	179 (97–287)	172 (127–213)	120 (75–177)	89 (53–142)	52 (36–72)	36 (23–54)	4·7 (2·8–7·8)	139 (92–192)
Arunachal Pradesh	597 (419–813)	300 (211–404)	155 (80–255)	148 (109–186)	109 (68–162)	110 (66–176)	54 (37–75)	40 (25–59)	5·3 (3·1–8·6)	119 (78–165)
Meghalaya	577 (405–784)	298 (208–402)	182 (98–294)	141 (104–178)	108 (67–161)	116 (69–185)	54 (37–75)	36 (23–53)	5·4 (3·2–8·7)	115 (75–159)
Karnataka	617 (431–838)	324 (229–434)	142 (74–234)	175 (131–220)	120 (75–180)	71 (42–116)	52 (35–72)	40 (25–58)	4·9 (2·8–7·8)	141 (93–195)
Telangana	756 (527–1025)	324 (228–434)	142 (75–232)	175 (130–218)	120 (76–178)	85 (51–136)	52 (36–72)	43 (28–64)	4·7 (2·8–7·5)	140 (92–193)
Gujarat	528 (372–716)	302 (214–407)	135 (70–222)	171 (126–215)	117 (74–171)	91 (55–144)	53 (36–74)	41 (25–60)	4·8 (2·8–7·8)	136 (89–188)
Manipur	616 (436–837)	360 (252–482)	184 (99–298)	162 (121–200)	118 (74–175)	96 (57–154)	53 (36–73)	33 (21–48)	4·9 (2·9–7·9)	133 (87–184)
Jammu and Kashmir*	475 (335–644)	312 (222–422)	168 (91–270)	160 (119–201)	117 (72–176)	106 (63–168)	54 (37–75)	36 (23–53)	5·2 (3·0–8·5)	130 (85–180)
Haryana	628 (440–851)	309 (219–415)	119 (61–198)	166 (124–207)	114 (72–169)	95 (57–151)	54 (36–75)	43 (27–64)	4·9 (2·9–8·0)	132 (87–183)
High SDI states (318 million population)	**651 (461–880)**	**329 (234–438)**	**121 (63–201)**	**181 (137–224)**	**120 (75–177)**	**84 (50–134)**	**51 (35–71)**	**42 (26–62)**	**5·2 (3·1–8·3)**	**144 (95–198)**
Uttarakhand	488 (346–666)	317 (224–426)	128 (64–213)	164 (122–204)	115 (73–171)	99 (60–158)	53 (35–73)	42 (27–62)	4·9 (2·9–8·0)	132 (88–183)
Tamil Nadu	836 (588–1123)	325 (230–434)	127 (67–210)	183 (137–228)	113 (71–170)	76 (45–121)	51 (35–70)	41 (26–60)	4·9 (3·0–7·9)	147 (97–202)
Mizoram	461 (326–633)	316 (223–423)	159 (84–260)	162 (122–203)	117 (73–175)	100 (60–160)	53 (36–74)	38 (24–57)	5·0 (3·0–8·2)	130 (84–179)
Maharashtra	626 (443–848)	324 (229–432)	127 (66–209)	178 (133–222)	121 (76–181)	90 (54–143)	53 (36–73)	43 (27–63)	6·2 (3·7–9·9)	142 (93–197)
Punjab	487 (348–666)	307 (217–413)	123 (63–204)	179 (133–224)	121 (75–181)	85 (50–135)	53 (36–73)	40 (25–59)	4·6 (2·8–7·5)	144 (94–198)
Sikkim	558 (395–762)	325 (228–439)	112 (56–188)	185 (136–231)	124 (78–183)	90 (53–144)	54 (36–75)	52 (33–77)	4·9 (2·9–8·0)	142 (93–197)
Nagaland	504 (353–684)	309 (216–414)	141 (72–236)	153 (113–193)	114 (71–170)	112 (66–179)	54 (37–75)	39 (25–57)	5·3 (3·1–8·6)	124 (81–172)
Himachal Pradesh	588 (415–803)	329 (234–440)	121 (63–201)	182 (136–227)	123 (77–186)	82 (49–130)	39 (26–54)	41 (26–60)	4·5 (2·6–7·2)	145 (95–199)
UTs other than Delhi	646 (458–884)	330 (234–444)	103 (51–176)	196 (147–244)	130 (81–194)	82 (49–131)	54 (37–75)	48 (31–71)	4·3 (2·6–6·9)	148 (97–205)
Kerala	641 (451–869)	383 (271–511)	107 (54–179)	192 (143–239)	132 (83–196)	71 (43–116)	47 (32–66)	38 (24–55)	3·3 (2·0–5·5)	149 (98–205)
Delhi	459 (324–621)	321 (226–432)	87 (42–146)	185 (136–230)	122 (76–183)	88 (53–141)	54 (37–74)	52 (33–77)	4·8 (2·8–7·9)	141 (93–194)
Goa	626 (441–848)	315 (221–423)	71 (32–124)	210 (155–262)	134 (85–199)	72 (43–115)	52 (35–71)	54 (34–80)	2·2 (1·2–3·9)	156 (103–215)

Table 2.2: Share of global population of various mental disorders.

Source: IHE, Global Burden of Disease study, 2017

Disorder	Share of global population with disorder (2017) [difference across countries]	Number of people with the disorder (2017)	Share of males: females with disorder (2017)
Any mental health disorder	10.7%	792 million	9.3% males 11.9% females
Depression	3.4% [2-6%]	264 million	2.7% males 4.1% females
Anxiety disorders	3.8% [2.5-7%]	284 million	2.8% males 4.7% females
Bipolar disorder	0.6% [0.3-1.2%]	46 million	0.55% males 0.65% females
Eating disorders (clinical anorexia & bulimia)	0.2% [0.1-1%]	16 million	0.13% males 0.29% females
Schizophrenia	0.3% [0.2-0.4%]	20 million	0.26% males 0.25% females
Any mental or substance use disorder	13% [11-18%]	970 million	12.6% males 13.3% females
Alcohol use disorder	1.4% [0.5-5%]	107 million	2% males 0.8% females
Drug use disorder (excluding alcohol)	0.9% [0.4-3.5%]	71 million	1.3% males 0.6% females

According to the 2016 National Mental Health Survey (NMHS), there was a distinct trend in the distribution of different mental morbidities among males and females (Figure 2.6). Males had higher prevalence rates for substance use disorders (F10-F19) and psychotic disorders (F20- F29), while females had higher prevalence rates for mood disorders (F30-F39) and neurotic & stress related disorders (F40-F48). Females had a significantly higher prevalence of depressive disorders, anxiety disorders, and eating disorders than males, while males had a significantly higher prevalence of conduct disorder, autism spectrum disorders, and ADHD. Mental morbidity increased with age in both classes (Figure 2.7). The prevalence rates for the majority of the disorders peaked in the 40-49 age bracket (NMHS 2016, Jayasankar et al., 2020).

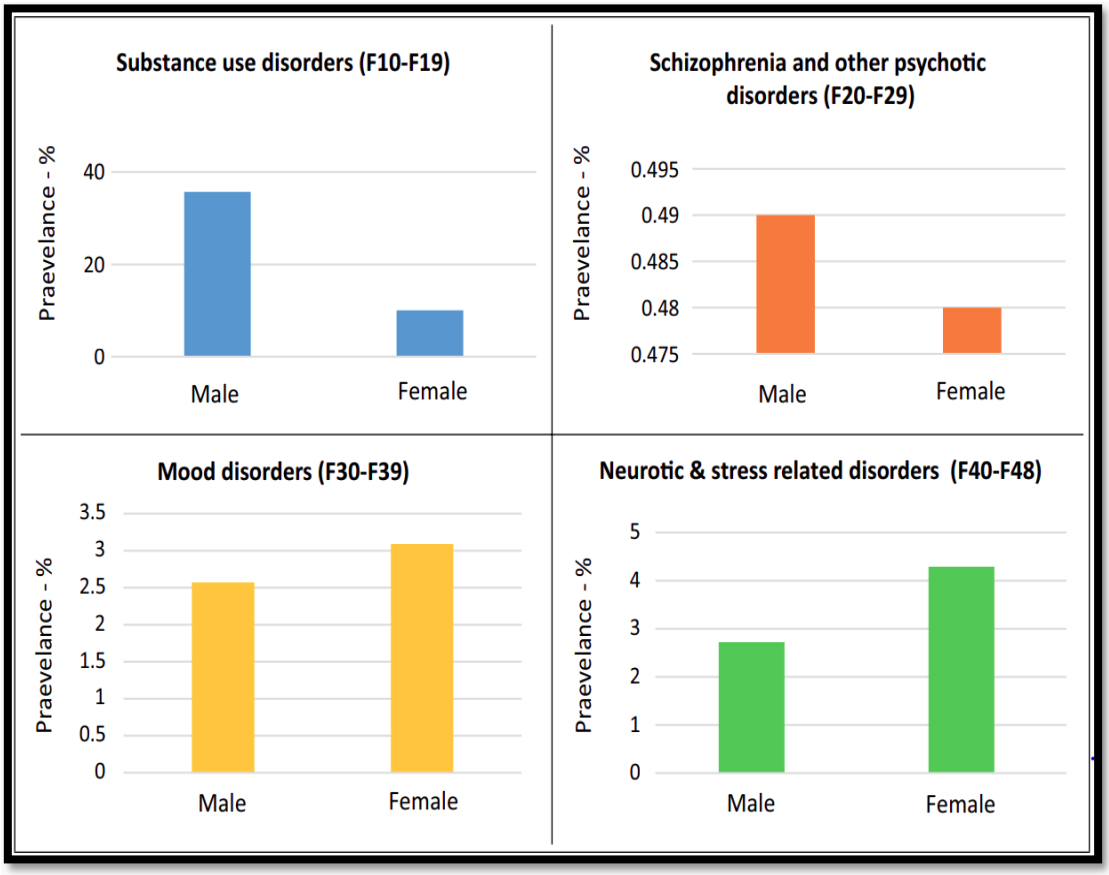

Figure 2.6: Distribution of various mental morbidities among males and females source: NMHS 2016

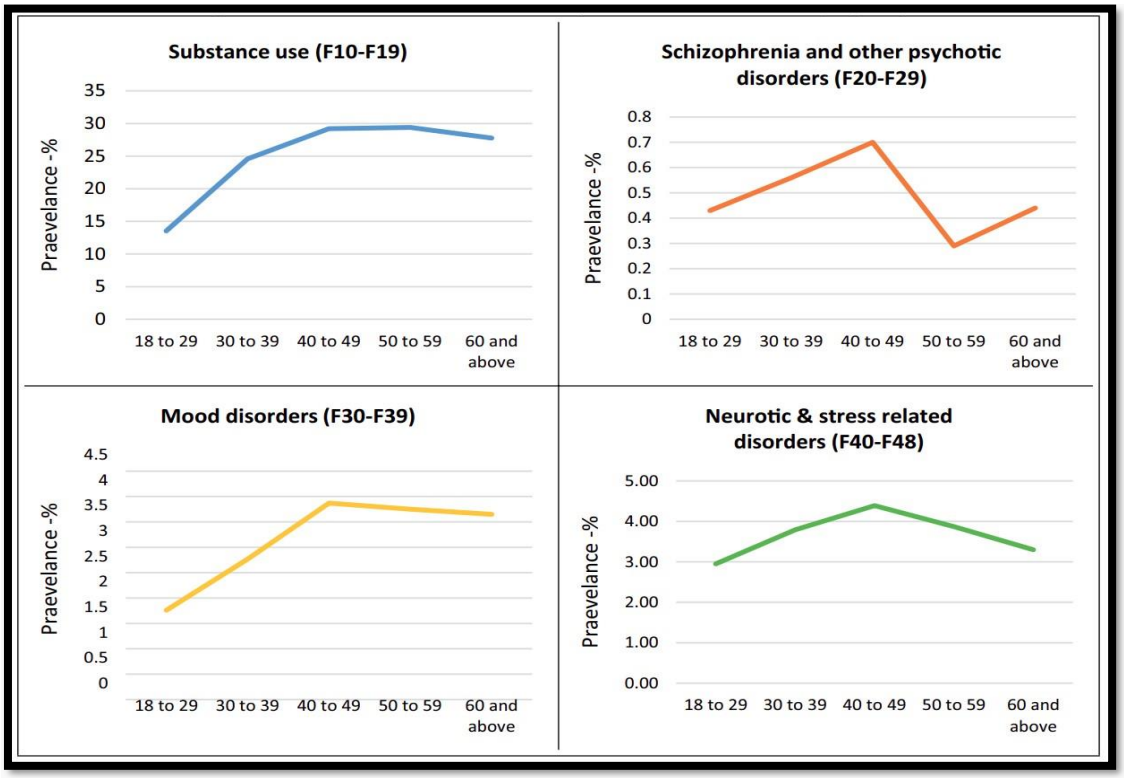

Figure 2.7: Mental morbidity rate across various age groups, NMHS 2016

Data from 2017 indicate that people most likely suffer from anxiety disorders followed by depressive disorders.

2.2 Incidence and Prevalence of depression

Depression is rising at alarming rate. Rates increased by one fifth from 2005 to 2015, Researchers have shown that people born after 1945 were ten times most likely to become the prey of depression than who were born before 1945. It is expected to be the world's leading cause of disability (James et al., 2020).

WHO estimates that more than 300 million people worldwide suffer from depression. The World Mental Health Survey conducted in 17 countries found that 1 in 17 people reported at least one episode of depression in the previous year (Mental Health Factsheet, WHO, 2012). By 2030, unipolar depression is predicted to be the second leading contributor to the global burden of disease first one being anxiety disorder (Mathers and Loncar, 2006).

According to the World Health Organization, nearly half of the depressive population lives in South-East Asia and the Western Pacific Region (Figure 2.8), which includes India. According to WHO global depression rates, the top ten most affected countries are China, India, the United States, Brazil, Bangladesh, Russia, Indonesia, Nigeria, Pakistan, and Iran. While the Solomon Islands, Papua New Guinea, Timor-Leste, Vanuatu, Micronesia, the Republic of Kiribati, the Kingdom of Tonga, Samoa, the Lao People's Democratic Republic, and Nepal are among the ten least depressed countries. (WHO, 2020).

According to the Global Burden of Disease Report (GBDS), which was conducted from 1990 to 2017 and published in 2020 as indicated in Table 2.3 depressive disorders (33.8 percent,) and anxiety disorders (19.0 %) were the leading contributors to DALYs attributable to psychiatric disorders in India in 2017, followed by IDID (10.8 %), schizophrenia (9.8 %), and bipolar disorder (5.9%) (James et al., 2020).

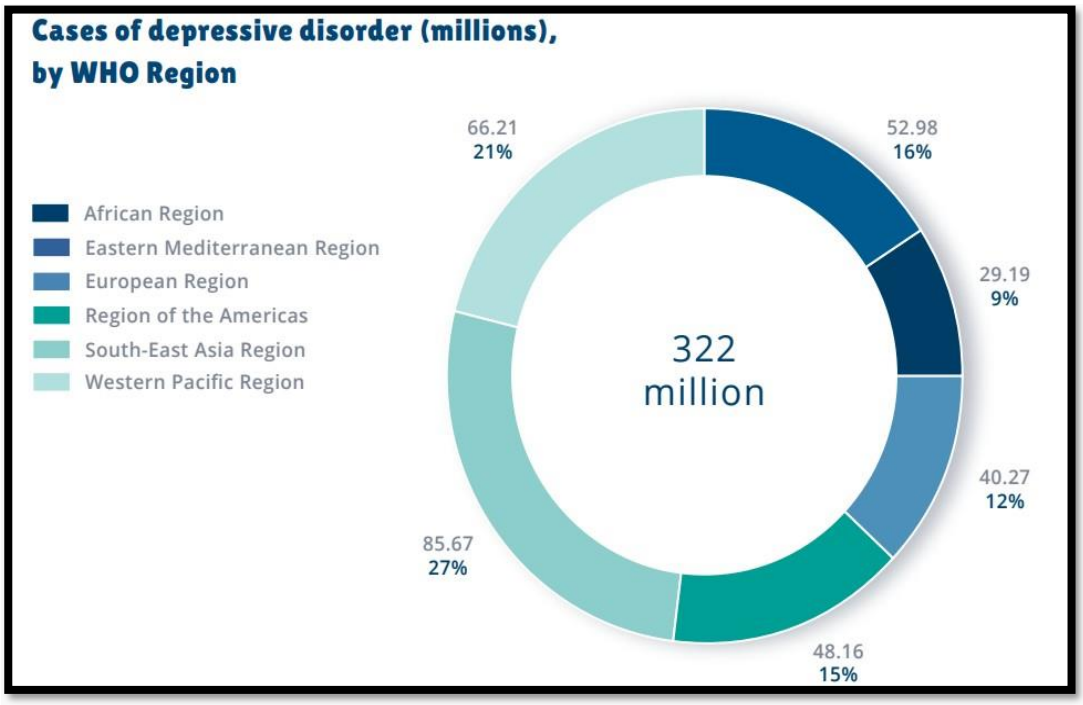

Figure 2.8: Region wise cases of depressive disorders, WHO 2020

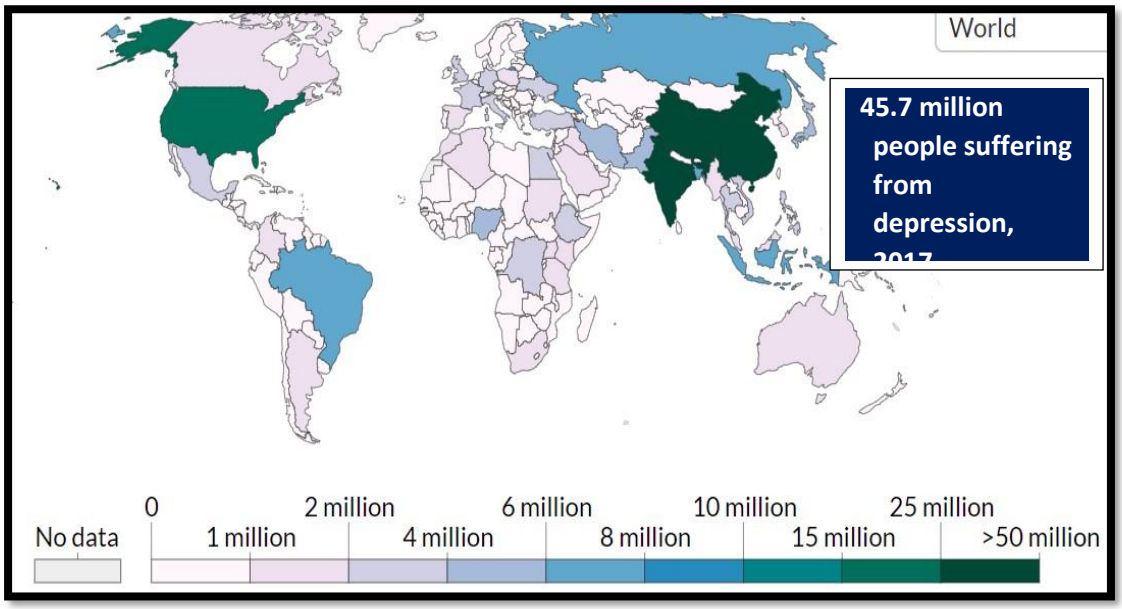

Figure 2.9: Share of people with depression across world and India. Source IHME, Global Burden of Disease, 2017

Table 2.3: Percentage of total DALYs due to each cause under mental disorder in India. Source: India State-Level Disease Burden Initiative Mental Disorders Collaborators, 2020

	Both sexes	Males	Females
Depressive disorders	33·8% (29·5–38·5)	28·9% (25·0–33·3)	38·6% (34·0–43·7)
Major depressive disorder	26·7% (22·6–31·2)	22·7% (19·0–27·0)	30·6% (26·1–35·8)
Dysthymia	7·1% (5·7–8·7)	6·2% (4·9–7·6)	8·0% (6·4–9·7)
Anxiety disorders	19·0% (15·9–22·4)	16·2% (13·5–19·2)	21·7% (18·1–25·5)
Idiopathic developmental intellectual disability	10·8% (6·3–15·9)	11·8% (7·0–17·4)	9·7% (5·6–14·6)
Schizophrenia	9·8% (7·7–12·4)	11·2% (8·8–14·0)	8·5% (6·7–10·8)
Bipolar disorder	6·9% (4·9–9·6)	7·2% (5·1–10·0)	6·6% (4·7–9·1)
Conduct disorder	5·9% (4·0–8·1)	7·9% (5·5–10·9)	3·9% (2·6–5·6)
Autism spectrum disorders	3·2% (2·7–3·8)	4·8% (4·0–5·7)	1·7% (1·4–2·0)
Eating disorders	2·2% (1·7–2·8)	1·5% (1·1–2·0)	2·8% (2·2–3·6)
Anorexia nervosa	0·5% (0·3–0·6)	0·2% (0·1–0·3)	0·7% (0·5–0·9)
Bulimia nervosa	1·8% (1·3–2·3)	1·3% (1·0–1·8)	2·2% (1·6–2·8)
Attention-deficit hyperactivity disorder	0·3% (0·2–0·5)	0·5% (0·3–0·7)	0·2% (0·1–0·3)
Other mental disorders	8·0% (6·1–10·1)	9·9% (7·5–12·4)	6·3% (4·7–7·9)

Data are percentage, with 95% uncertainty interval in parentheses. DALYs=disability-adjusted life-years.

2.2.1 The burden of depression across the states of India

The Global Burden of Disease Study 1990–2017 pointed that in 2017, 45·7 million people had depressive disorders in India. Prevalence of depressive disorders varied 1·9 times among the states, with the highest prevalence observed in Tamil Nadu, Kerala, Goa, and Telangana in the high Socio-demographic Index (SDI) state group; Andhra Pradesh in the middle SDI state group; and Odisha in the low SDI state group. As reported by NMHS, 2016 (Jayasankar et al., 2020) among the states, prevalence of current DD varied from 1.2% in Gujarat to as high as 4.7% in Jharkhand. Tamil Nadu (4.5%), West Bengal (4.3%) and Manipur (3.7%). Figure 2.10 represents Tamil Nadu, which is classified as a high SDI state with an SDI of 836, had the highest DALY rates due to depressive disorders in 2017. Furthermore, Andhra Pradesh, a state with a medium SDI, had a DALY rate of 793. In comparison, Bihar, which has a low SDI of 406, had the lowest DALY rate in relation to depressive disorder (Statista Department, 2022).

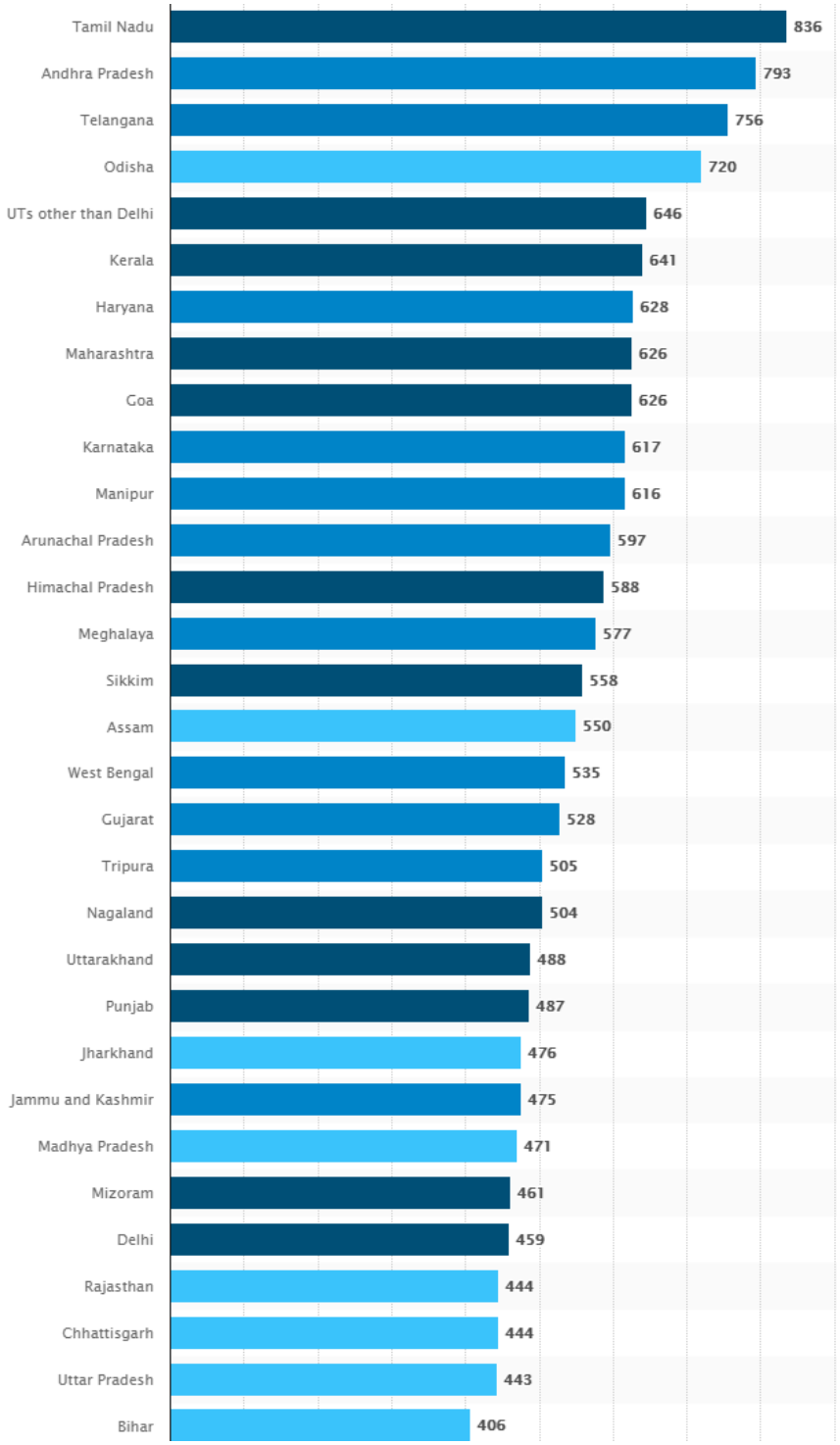

Figure 2.10: DALYs relative to depressive disorders across India, 2017
Source: Statista Department

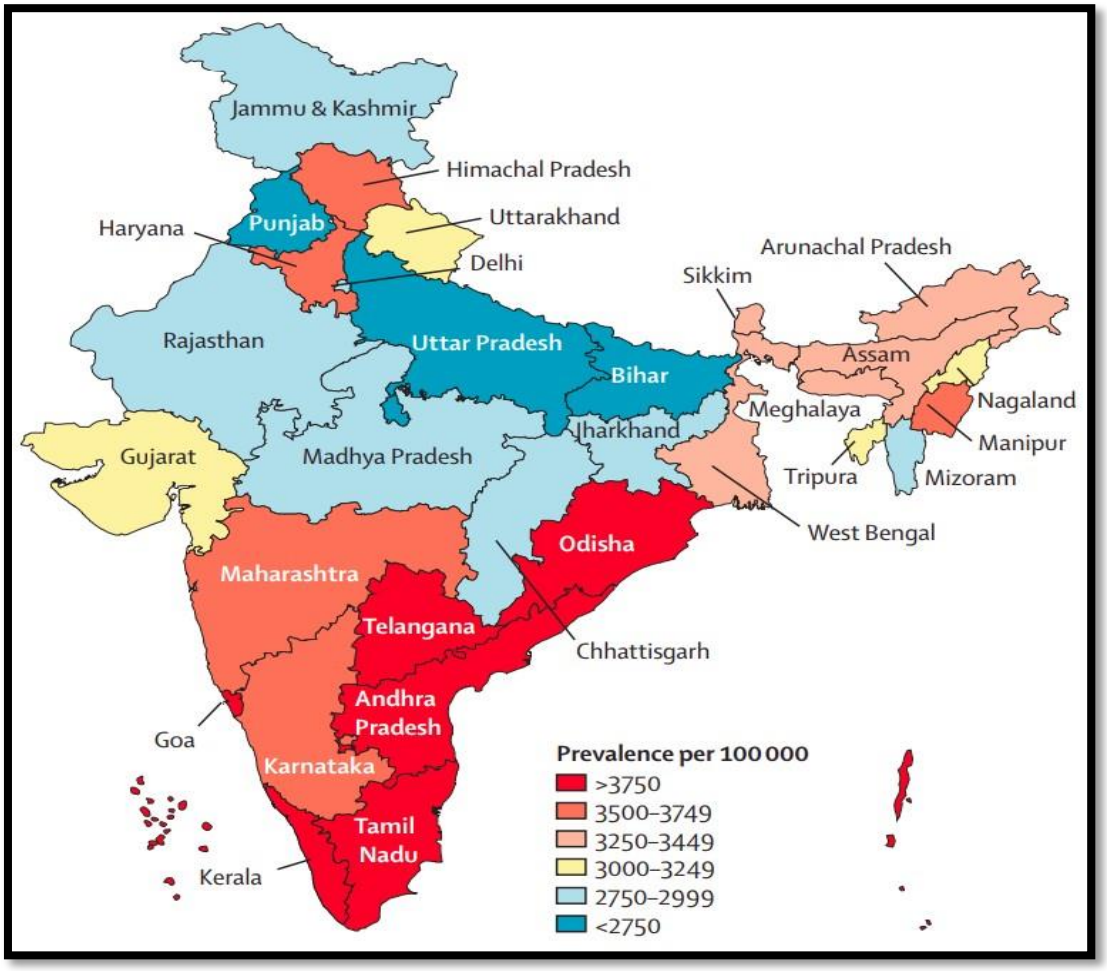

Figure 2.11: State wise crude prevalence of depressive disorders in India

2.2.2 Gender wise prevalence of depression

Overall rates of depression were found higher in females in compression to males. WHO global health estimates 2015, showed that in all the regions females were more depressed. The prevalence of depressive disorders at its worst is positively associated with the suicide death rate. At its worst, depression can lead to suicide. Close to 800 000 people commit suicide every year making it the leading cause of death in 15-29-year-olds (WHO factsheet, 2020). At the state level for both sexes, there is slightly higher correlation coefficient in females ($r=0{\cdot}57$, $r^2=0{\cdot}33$; $p=0{\cdot}0009$) than in males ($r=0{\cdot}44$, $r^2=0{\cdot}19$; $p=0{\cdot}015$) (GBDS, 2020). Figure 2.11 and Figure 2.12 specifies number of males and females with depression across the world and in India respectively. In Figure 2.13, gender wise prevalence of depressive disorder in WHO region is indicated.

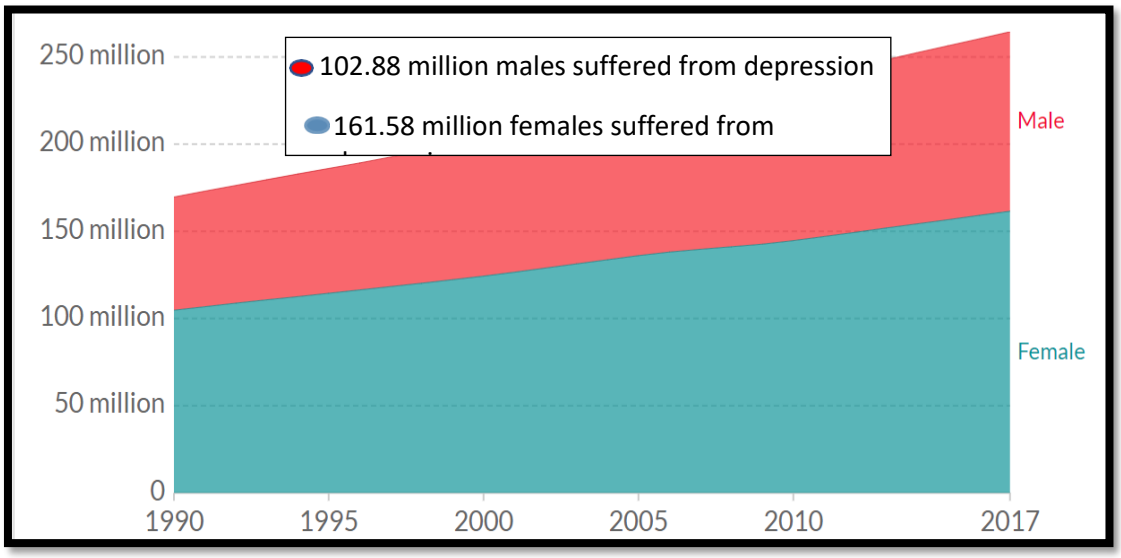

Figure 2.12: Number of people with depression, World 2017: IHME, Global Burden of Disease 2017

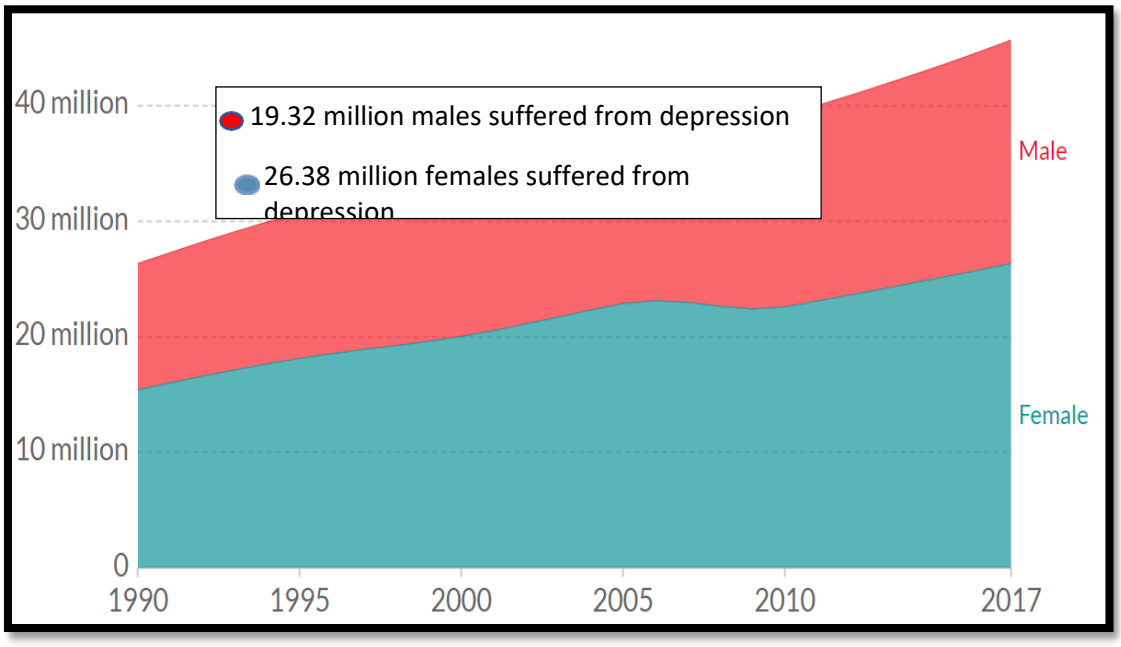

Figure 2.13: Number of people with depression, India 2017: IHME, Global Burden of Disease 2017

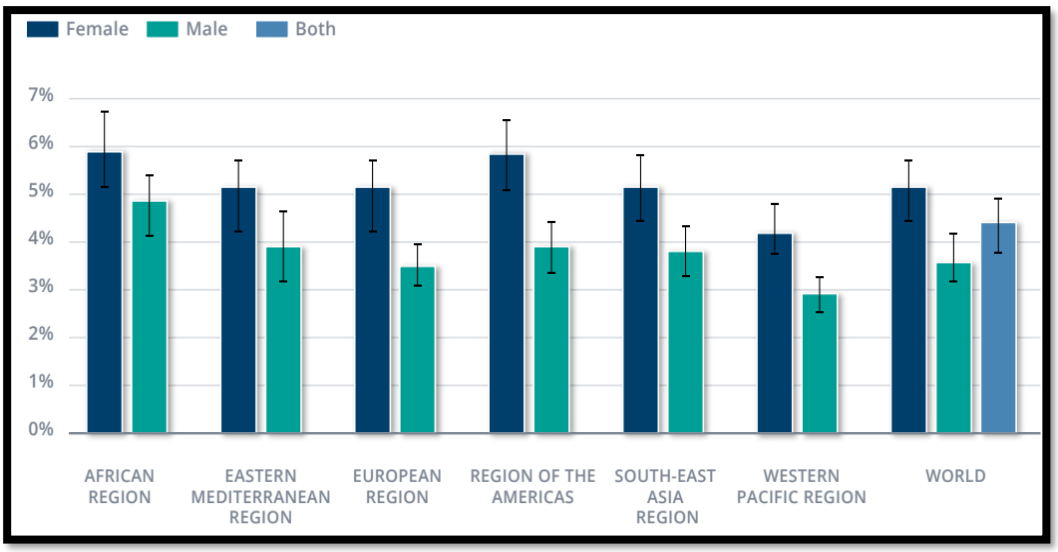

Figure 2.14: Prevalence of depressive disorders by WHO region

Depression is the most common cause of disability in the world. It causes more 'years lost to disability than any other disease on a global scale (National Institute of Mental Health).

2.3 History of depression

Depression is a significant human affliction. It refers to a group of diseases, disabilities and episodes that are frequently debilitating in nature, range in severity from mild to extreme, and last for months to years. It is a severe health problem that can begin any age and last for a long time, once treated it can reoccur. The whole series of contributors can be credited for the identification of depression as a clinical condition affecting both physiological and psychological responses. Clinical depression was earlier known as melancholia (Hippocrates, 1923).

Hippocrates a Greek physician (460 BCE–370 BCE) introduced the theory of the four humors—blood, yellow bile, black bile, and phlegm, any disturbance in mental or physical health were linked to irregularity in one of these. Greeks believed melancholia occurred due to excessive black bile secretion in the body (Liddell and Robert, 1980).

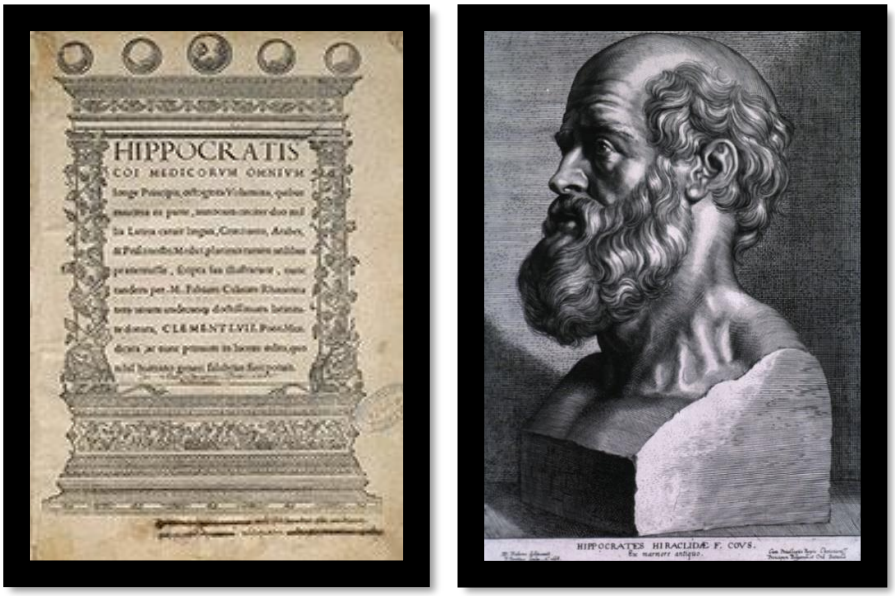

Figure 2.15: Hippocrates, *De Humorous*, 1525. Courtesy National Library of Medicine

Melancholia was defined in the fifth century BC by Hippocrates as: ***"Fear or sadness that last a long time"*** (Hippocrates, 1923). Its manifestation was marked remarkably similar to the current definition of depression found in the Diagnostic and Statistical Manual of Mental Disorders (DSM–5) as "clustering of the symptoms of sadness, dejection, despondency, fear, anger, delusions obsessions, aversion to food, sleeplessness, irritability, and restlessness" (American Psychiatric Association, 2013).

Galen conducted a case study on melancholia patients and came to the conclusion that he couldn't tell whether the patients were dull or strict for no obvious reason or whether there was some underlying explanation for their depressive behaviour (Radden, 2003). Inflammation in the meninges of the brain was known as phrenitis often attended with acute fever and delirium. Ishaq ibn Imran through his work, The Canon of Medicine clubbed the concepts of melancholia and phrenitis (Morelon and Rashed, 1996).

Hippocrates, Galen, and Ishaq's work gradually came to be regarded as the gold standard of medical thought in Europe. (Abbasi et al., 2007). Around the 17th century, Robert Burton published The Anatomy of Melancholy, a magisterial work that argued that patients experience "sorrow and grieving without any apparent cause." His work also mentioned that melancholy can be alleviated by following a balanced diet, getting enough sleep, listening to music, and
engaging in social activities (Berrios, 2016).

Figure 2.16: ***The Anatomy of Melancholy*** **(Left) published by Robert Burton (Right)**

Johann Christian Heinroth argued in the 18th century that the word psychosomatic should be used for psychiatric disorders rather than melancholia because he believed the latter was associated with soul disruption and should not be confused with physical wellbeing (Jackson, 1983).

Louis Delasiauve coined the word "Depression" in 1856, which means "to press down." By 1860, the word was being used in medical dictionaries to describe physiological and metaphorical declines in emotional activity (Berrios, 1988). From the 17th to the 19th centuries, depressive disorders were divided into two categories, each with its own care. Patients with persistent feelings of mental anguish, hopelessness, joylessness, stupor, and suicidal thoughts were treated in the first condition. Healers who tended delusional patients were referred to as "alienists" at the time. The second group stressed the importance of nerves fibers, and organs in mental stability, labelling any systemic disturbances as "nervous disorders." They concluded that since the diseased condition was caused by nervous system abnormalities, it was not a case of mental stability and that it should be investigated by physicians or neurologists (Horwitz et al., 2015).

Kraepelin in 1903 studied the underlying brain pathology in depression. He advocated for the distinction between endogenous (inner) and exogenous (external) depression (Davison, 2006).

Kurt Schneider, a German physician, invented the terms endogenous depression and reactive depression in 1920. Endogenous depression has no external cause. The root cause for this is genetics or biological factors. In today's terms, this is known as Mild Depressive Disorder (MDD). “Reactive” depression arises when a person goes through a traumatic or painful period of his life, which leads to depression (Malki et al., 2014).

Sigmund Freud, the father of psychoanalysis, said that depression, or melancholia, is a more extreme form of grief than mourning. The id, ego, and superego, according to Freud, are constantly at odds, and adult personality and actions are shaped by the outcomes of these internal conflicts during childhood. He believed that an individual with a strong ego has a healthy personality, and that ego imbalances can contribute to neurosis (what we now call anxiety and depression) and unhealthy behaviour (Rhee, 2017).

In the mid-20th century, various psychologists came forth with proposals relating depression with different factors. The "father" of behaviourism, John B. Watson, founded behaviourist theory. Behaviourism was the prevalent school of thought in psychology from 1920 to the mid-1950s. It emphasises that an individual's behaviour, including depression, is shaped by the environment to which he is exposed. Classical conditioning and operant conditioning are the two main forms of conditioning, according to behavioural psychology. Depression, according to classical conditioning, is a learned behaviour in which certain negative stimuli are correlated with emotional states. The removal of positive feedback from the community causes depression (Le winsohn, 1974). Losing a career or a loved one, for example, reduces positive reinforcement.

Viktor Frankl Austrian existential psychiatrist linked depression to feelings of futility and meaninglessness (Frankl, 2000). "Depression is the inability to create a future," Rollo May proposed (Geppert, 2006). Depression, according to Abraham Maslow, is more likely to occur when the universe denies the self-actualizer a sense of "richness" or "totality" (Maslow, 1971).

Aaron Beck, a cognitive theorist, established the depression-causing mechanism of the cognitive triad in 1967. Individuals establish a pessimistic critical thinking

pattern about themselves, the environment, and the future, which leads to depression. These negative schemas, according to Beck, are formed in childhood as a result of a traumatic event (Beck, 1979). The effects of reserpine and isoniazid on monoamine neurotransmitter levels and depression were studied in 1950. Depression was discovered to be the product of a chemical imbalance of neurotransmitters (Schildkraut, 1965).

2.4 Symptoms of depression

Sadly, up to 80% of depressed people have some form of impairment in their daily functioning (Pratt and Brody, 2008). The Diagnostic and Statistical Manual of Mental Disorders has been revised five times between 1952 and 2013.

All forms of depressive disorder experience some of the following symptoms:

Feeling sad or having a depressed mood

Loss of interest or pleasure in activities once enjoyed

Changes in appetite — weight loss or gain unrelated to dieting

Trouble sleeping or sleeping too much

Loss of energy or increased fatigue

Increase in purposeless physical activity (e.g., hand-wringing or pacing) or slowed movements and speech (actions observable by others)

Feeling worthless or guilty

Difficulty thinking, concentrating or making decisions

Bleak and pessimistic views of the future;

Ideas or acts of self-harm or suicide

Reduced self-esteem and self-confidence

Symptoms should last for at least two weeks for the diagnosis of depression (National Institute of Mental Health).

2.5 Depression and its types

The four most common types of depression are Major Depression, Persistent depressive disorder (dysthymia), bipolar disorder, and seasonal affective disorder. Major depression: It is the most common type of depression. It is characterized by a dark mood that consumes one's life and causes one to lose interest in activities, even those that are normally pleasurable. Marked by disruption in sleeping and eating patterns and always lowon energy. Suicidal or death thoughts may arise.

Persistent depressive disorder(dysthymia): Refers to low mood disorder that last for as long as two years but is not severe in intensity. Patient is usually distressed by the symptoms and has some difficulty in continuing with ordinary work and social activities, but will probably not cease to function completely. Depressed mood, loss of interest and enjoyment, and increased fatiguability are usually regarded as the most typical symptoms of depression,

Bipolar disorder (manic-depressive disease): symptoms are opposite of depressive symptoms people are usually hyperactive grandiose ideas, unrealistically high self-esteem, decreased need for sleep, thoughts and activity at higher speed, and ramped-up pursuit of pleasure including sex sprees, overspending, and risk taking. If not stabilized overtime leads to self-destructive behavior, and is usually followed by a period of depression.

Seasonal affective disorder (SAD): Seasonal depression manifests itself as the days get shorter in the fall and winter. Changes in the body's normal daily cycles, the eyes' sensitivity to light, or the role of chemical messengers like serotonin and melatonin may all contribute to a shift in mood. The most common treatment is light therapy, which entails sitting in front of a very bright light source for regular sessions.

Depression types unique to women

Researches have clarified that woman are at higher risk for general depression, but they are also prone to two different depression types that are influenced by reproductive hormones

1) **Perinatal depression (postpartum depression):** occurs during pregnancy or within the first year after delivery. Both major and minor depressive episodes are involved. One out of every seven pregnant women is affected by this condition. Family should be supportive to prevent devastating effects on the women, their infants, and their families.

2) **Premenstrual dysphoric disorder (PMDD):** Premenstrual syndrome, or PMS, is a serious form of depression. PMDD symptoms normally appear soon after ovulation and disappear once menstruation begins. Selective serotonin reuptake inhibitors like fluoxetine (Prozac) and sertraline (Zoloft) can help alleviate symptoms (WHO factsheet, 2020).

2.6 Measuring depression

Blood, urine and stool testing can easily be used as indicative of various chronic illness like diabetes, thyroid, cholesterol levels etc. But unfortunately, we don't have any invasive test available to know specific percentage of depression severity. And this is the root cause behind several depressive disorders being left untreated, which is life threatening. We are dependent on symptoms and signs for diagnostic hypotheses and they have proved out to be quite relevant. For measuring the levels of depression standardized age specific tools have been designed. These screening tools should have high sensitivity (few false-negatives), good content validity (do they measure what we think they do), test-retest reliability (reliability over time), good inter-rater reliability (agreement between clinicians), and high specificity (fewer false- positives). Current available instruments used for screening, diagnosis and tracking of depression includes both interview and self-report measures.

2.6.1 Inventory scales for depression analysis

The Centre for Epidemiologic Studies Depression Scale (CES-D) is a 20 items self-report questionnaire designed for measuring major dimensions of depression in the general population and can be used for children aged 6 and above. The responses are scored on a 4-point scale. It has been tested across gender and cultural populations and has reported well-maintained consistency validity and reliability. The scale takes about 20 minutes to administer, including scoring (Saracino et al., 2018).

The EQ-5D was introduced by a group of European researchers to measure health-related quality of life. It uses five dimensions mobility, self-care, usual activities, pain/discomfort, and anxiety/depression to measure the quality of life. time taken for administering the questions is less than five minutes. There are different versions for adults (age 16 and older) and children EQ-5D-Y (8 to 15 years). The EQ-5D is used worldwide in wide range of languages (Devlin and Brooks, 2017)

The Hamilton Rating Scale for Depression, (HRSD) is generally used by health care professionals to measures depression in individuals before, during, and after treatment.The scale contains 21 items but is scored based on the first 17 items, measured either on 5-point or 3-point scales. time taken for administration is 15 minutes (Trajković et al., 2011).

Montgomery-Åsberg Depression Rating Scale (MADRS) a 10-item rating scale is an adaptation of the Hamilton Depression Rating Scale which measures the severity of depression in individuals over 18 years. Each item is rated on a 7-point scale. The scale has a greater sensitivity to change over time and generally takes 20 to 30 minutes to assess (Montgomery and Åsberg, 1979).

The Social Problem-Solving Inventory (SPSI) is a self-report measure that takes 10 to 20 minutes for the administration of social problem-solving strengths and weaknesses in individuals 13 years old and older. The scale has both long-form (52 questions) and short-form (25 questions) (D'Zurilla et al., 2002).

Quick Inventory of Depressive Symptomatology-Self-Report (QIDS-SR) is another popular self-assessment scale for assessing the severity of depression in adults 18 years and above. There are 16 measures, selected from the Inventory of Depressive Symptomology. A 4-point Likert-type scale assesses the behaviors and mood over the course of the past week. It takes five to seven minutes to complete the report (Rush et al., 2003).

The Reminiscence Functions Scale (RFS) 6-point Likert-type scale is a 43-item questionnaire that takes 15 to 25 minutes to complete and the responses are scored in eight different categories. It assesses the frequency with which adults, 18 years and older, are able to recollect past experiences or events based on which depression is determined (Robitaille et al., 2010).

The Social Adjustment Scale (SAS-SR) is 54 items rated on a 5-point scale to measure the self-report of social functioning in 20 minutes for individuals above 17 years (Gameroff et al., 2012).

The Social Functioning Questionnaire (SFQ) is self-administrable and takes less than four minutes to measures social functioning in adults The questionnaire contains eight questions which are rated on a 4-point scale (Tyrer et al., 2005).

The Patient Health Questionnaire (PHQ) sets are rating scales, to assess the presence of depressed mood and loss of interest or pleasure in routine activities which person can report by themselves. It has two versions- the Patient Health Questionnaire-9 (PHQ-9) having 9-question for evaluation of Mental Disorders (Whooley et al., 1997). Another one is Patient Health Questionnaire-2 (PHQ-2) a shorter version requiring only one to five minutes with only two screening questions (Martin et al., 2006).

The Beck Depression Inventory (BDI) is the most appropriate and widely used tool to screen and measure depression behavioural manifestations and severity. It was developed by Aaron T. Beck, Ph.D., Center for Cognitive Therapy, Philadelphia, A. Beck and colleagues studied outpatient samples that included persons with severe psychiatric diagnoses, depressive disorders, substance abuse, and college students. BDI has been widely accepted and used in psychology and psychiatry for assessing the intensity of depression in psychiatric and normal populations. The tool can be used for the assessment of disease in the age group of 13 to 80. The inventory contains 21 self- report items that reflect the cognitive, affective, somatic, and vegetative symptoms of depression in multiple-choice response formats. Self-administration takes 5-10 minutes while Oral ministration uses 15 minutes. BDI is reliable and has high internal consistency from .73 to .92 with a mean of .86, with alpha coefficients of .86 and .81 for psychiatric and non-psychiatric populations, respectively (Richter et al., 1998) carried meta-analysis of studies on the BDI's psychometric properties reporting high content validity, in differentiating between depressed and non-depressed people (Beck and Garbin, 1988) reported that the BDI has been found to include three to seven factors including factors that reflect negative attitudes towards self, performance impairment, and somatic disturbances, as well as a general factor of depression.

The BDI has gain so much popularity that it has been translated into several languages including Spanish, Chinese, Dutch, Finnish, French (Canadian), German, Korean, Polish, Swedish, and Turkish. Due to high reliability and validity BDI is among most extensively used tool for screening depression. The prevalence of depressive and anxiety symptoms among medical students in an international medical university in the Kingdom of Bahrain was assessed using Becks (Mahroon et al., 2018). Depression among medical, dental, and engineering students in Patna was screened using Beck's Depression Inventory-II (Nezam et al., 2020).

Inventory scales used for older adults

The Geriatric Depression Scale (GDS) is specially designed in such a way that it is easy to assess the cognitive dysfunction of older adults. It contains 30 forced-choice "yes" or "no" questions relating to how an individual has felt in a specified time frame. It takes 10 minutes to administer GDS (Lopez et al., 2010).

Life Satisfaction Ratings (LSR), designed to measure well-being and successful aging among adults over the age of 50 is administered only by a health care professional. There are five categories that are rated on a 5-point scale. The estimated time for completing the questionnaire is 10 minutes. (Neugarten et al., 1961; Barrett and Murk 2006).

2.6.2 Biomarkers for depression assessment

Questionnaires and other standardized tools have proved to be reliable means for depression prognosis. Although, there is no laboratory based diagnostic tests for this disorder. Still the exploration of biomarkers in screening holds more authenticity. The scope of using biomarker underlies in the fact that the cause effect relationship of biochemical changes can be made out. Biomarkers has revealed association of depression with gastrointestinal factors (Wallace and Milev, 2017) endocrinology including neurotransmitters (Andrews et al., 2015) neurotrophic factors (Duman, 2012) hormones (Holsboer, 2000) and oxidative stress (Black, 2017). Patients with MDD in particular have shown disturbances in several neurotransmitters like dopamine, glutamate, γ-aminobutyric acid (GABA), and serotonin in the periphery and brain (Zheng et al., 2016).

Cortisol as a stress biomarker

In major depression and anxiety, hypercortisolemia is one of the most reliable biological readouts. Although the hypothesis supports cortisol hyperactivity in depression, hypoactivity has been observed in other illnesses such as panic disorder. Other factors also influence cortisol secretion. The neuroendocrine system, which includes the hypothalamus, pituitary gland, and peripheral endocrine glands, facilitates communication between the brain and the gut (Sudo, 2014; Rieder et al., 2017; Cussotto et al., 2018). The hypothalamic–pituitary–adrenal (HPA) axis is the primary neuroendocrine transmission pathway and a critical component of the stress response system. Neurons in the hypothalamic paraventricular nucleus (PVN) release two neurohormones, CRF and arginine vasopressin (AVP), into the blood vessels that connect the hypothalamus and pituitary gland (i.e., hypophysial portal blood). CRF binds to CRF receptors on the anterior pituitary gland, causing the protein proopiomelanocortin to be secreted (POMC). POMC stimulates the release of stress-related hormones such as adrenocorticotropic hormone (ACTH), -lipotropin (-LPH), and -endorphin. ACTH, in turn, stimulates the adrenal release of cortisol, a well-known stress biomarker hormone (Scheuer et al., 2012).

Positron emission tomography (PET) scan

PET is an imaging test which helps reveal how our tissues and organs are functioning. Figure2.17, shows comparative brain activity between normal and depressed brain, an increase of blue and green colors, along with decreased white and yellow areas, in brain activity due to depression.

Figure 2.17: A PET scan comparing brain activity during periods of depression (left) with normal brain activity (right). Brain scans Stanford Medicine, Accessed 2021

Nearly 15% of Indian adults (those above 18 years) are in need of active interventions for one or more mental health issues NMHS 2015-16. The disabilities and impact are ominous and affect, work, family and social life. However, to address these problems, the current mental health solutions are fragmented and uncoordinated. There is need to focus on less explored solution, which is proving to be one of the effective modes for depression management answer lies in the very primitive form of life on the planet: 'Microbiome'

2.7 Unmasking the Invisible World: The Microbiome

The first life to colonize this planet were microbes, which dates back to around 3.6 billion years. It is the origin of most organisms while the human began roaming the earth a mere 200,000 years ago (Cardona, 2018). Figure 2.18 depicts the emergence of different organisms at various times in the history of planet earth. Figure: 2.19 shows Use of microbes in ancient civilizations. Microbes were an integral part of wine- making; agriculture and curing disease.

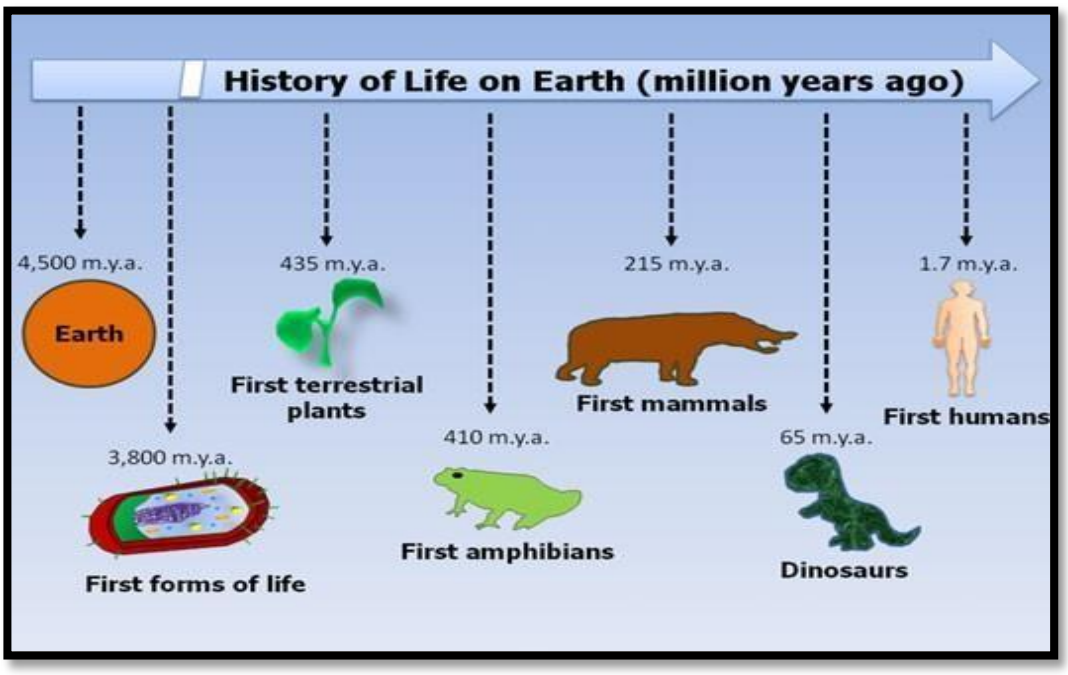

Figure 2.18: Life on Earth – A chronological history. The emergence of different organisms at various times in the history of planet earth

The term '*Adrista'* to describe the invisible world linking to microscopic creatures around us knows as *krimi* in Veda was first mentioned in the ancient Sanskrit scripture around 1500– 1200 BC (Jakhmola, 2010). Ebers Papyrus (1500 BC) describes various treatment modalities using natural resources as mud, oils, vegetables and plant extracts to cure ailments in belief that these possess magical powers to heal. In ancient China and Greece, moldy bread was utilized to prevent infections. Though they were not aware about microorganism present. Microbes and their secretions may have been the architect of these remedies (Davies, 2012).

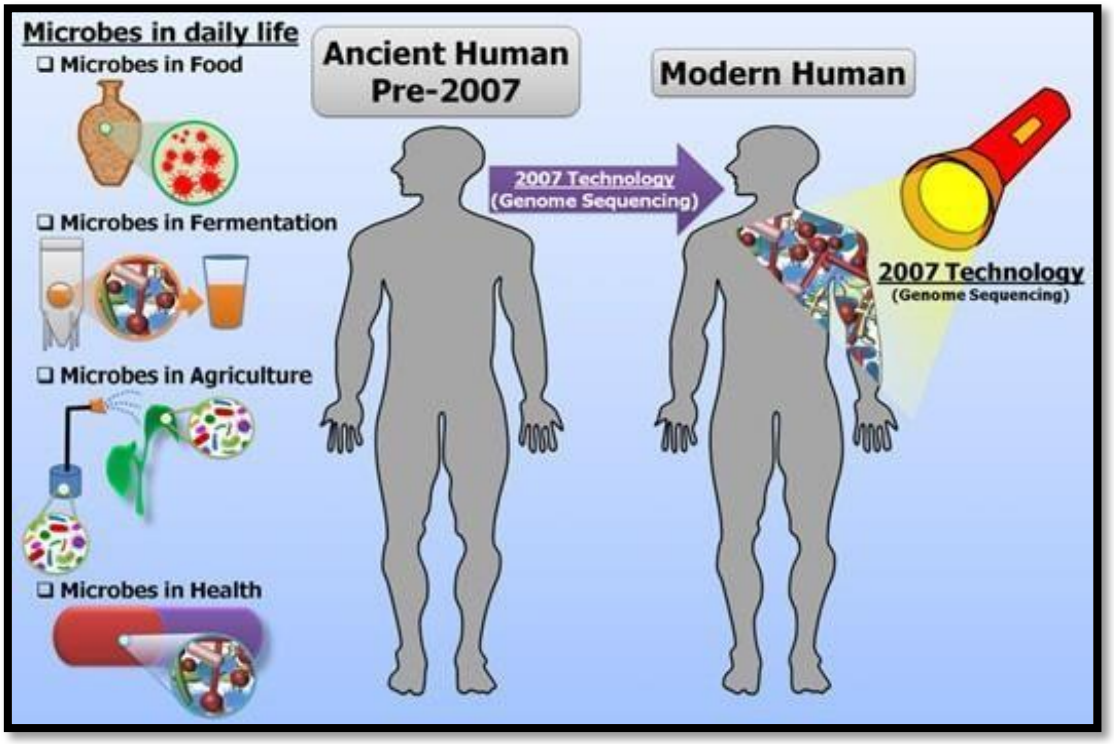

Figure 2.19: Use of microbes in ancient civilizations.

According to a letter from Antonie van Leeuwenhoek dated September 12, 1683, he studied his own microbiota in the 1680s. In both stable and diseased conditions, striking variation in oral and faecal habitat was discovered. To assess their number, he used single lens microscope and labelled the cells as "animalcules," which are now known as unicellular organisms. Robert Koch discovered in 1876 that microorganisms can cause disease. He discovered that anthrax infected cattle's blood contained a significant number of Bacillus anthracis. A slew of disease-causing microbes was discovered as a result of this frantic search. The major microbial causes of diseases like smallpox, polio, tuberculosis, and cholera have been identified. Louis Pasteur, a French scientist, demonstrated the role of these microorganisms in fermentation in 1877 (O'Hara and Shanahan, 2006). Overtime the endosymbiotic relationship of microorganisms with humans were revealed (Bukharin et al., 2012). The discovery of the microscope in 1635 allowed to see the invisible world made up of microbes. a Good (*Lactococcus lactis* in dairy products). b The Benign (*Pseudomonas fluorescens*-free living in the soil). c Deadly (*Bacillus anthracis*-lung disease).

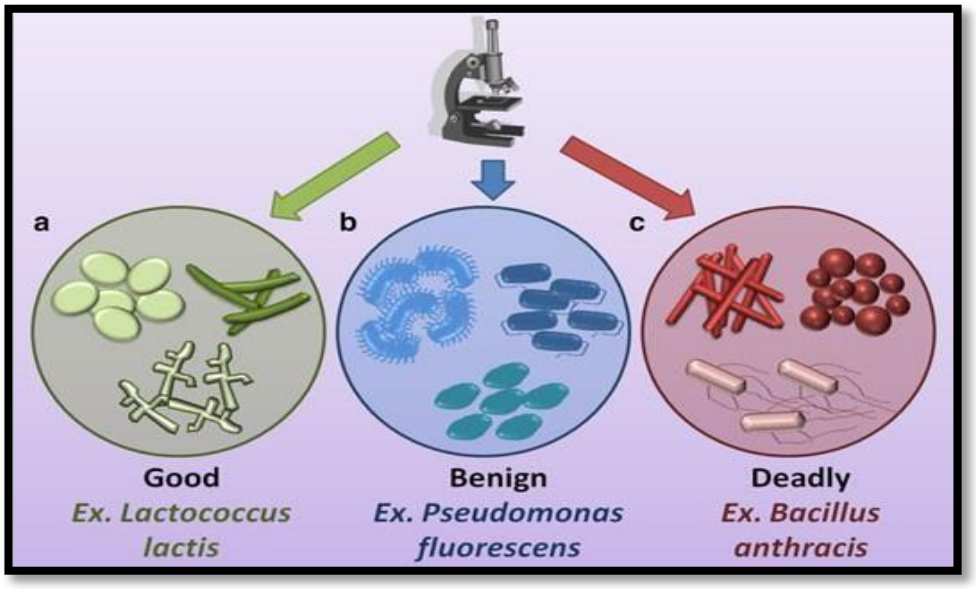

Figure 2.20: The visualization of microbes (the good, the benign and the deadly)

2.7.1 Glimpse at human gut microbiota

Live bacteria account for around 2 lbs. of a body's weight (Karlsson, 2013). The human gastrointestinal tract is highly dynamic with mucosal surface area of 300 m^2. It comprises, series of complex organs ranging from the stomach to the distal colon which harbours approximately 1014 microbial cells which is 10 folds higher than the number of human cells present in our body (Sekirov et al., 2010). There are almost 50 phyla (family) of bacteria (Figure 22). The most common phyla are the *Firmicutes, Bacteriodes, Proteobacteria, Actinobacteria,Fusobacteria and Verucomicrobia* (Rinninella et al., 2019).

Firmicutes thrive in the small intestine's proximal end. This territory is not suitable for *Bacteriodetes* colonisation; however, the distal region (colon and caecum) is ideal for *Actinobacteria* and *Proteobacteria* family members. The presence of a layer in the colon is rich in complex carbohydrate-like mucin which allows microbes to colonize. Dysbiosis of the gut microflora is often associated with bacterial translocation mainly due to intestinal barrier failure (Putignani, 2014) which promotes overgrowth of pathogenic bacteria such as *Salmonella, Shigella, Clostridia, Staphylococcus aureaus, Candida albicans, Campylobacter jejuni, Escherichia coli, Veillonella,* and *Klebsiella* which secrete proinflammatory cytokines amyloids and lipopolysaccharides, associated with the pathogenesis of

various metabolic disease and infections affecting liver, respiratory tract, gastrointestinal cancers and neurodegenerative diseases such as AD and depression (Wang et al., 2017; de et al., 2018).

On contrary, increased density of good bacteria, particularly *Bifidobacteria* and *Lactobacilli* are marker of the stable gut microflora. They produce strong acids, i.e., butyric acid, acetic and lactic acid which reduces intestinal pH and neutralizes the activity of dietary carcinogens, such as nitrosamines inhibiting the growth of pathogens preventing several diseases (Slavin, 2013; Tojo et al., 2014). Figure 2.22 and Figure 2.23 depicts Microbial diversity (good and bad) in human gut (Tojo *et al.*, 2014).

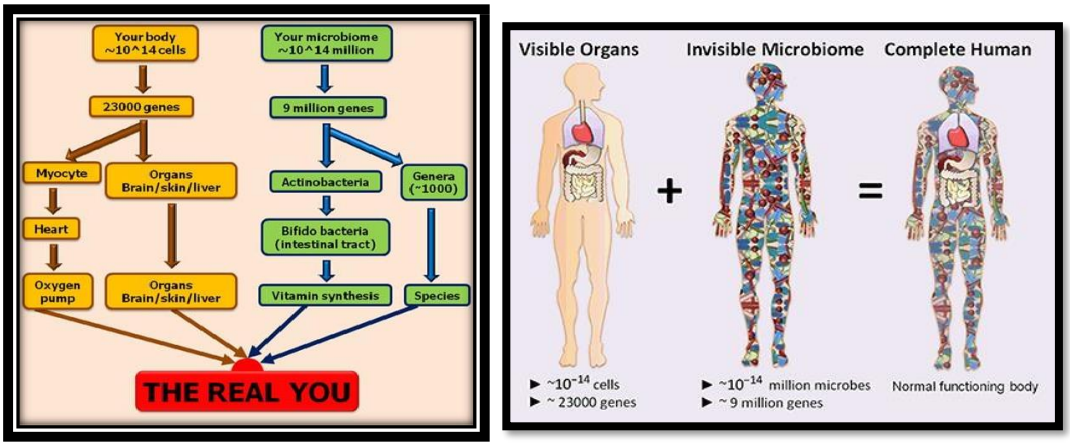

Figure 2.21: The real you with the visible and invisible constituents

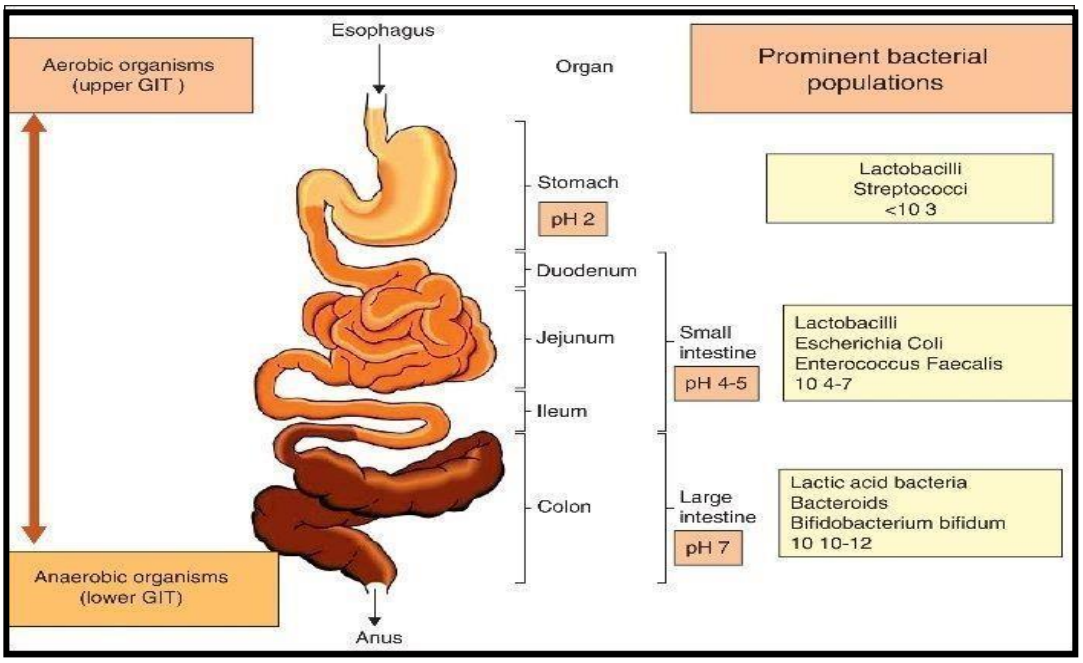

Figure 2.22: Prominent Bacterial population within the human gut

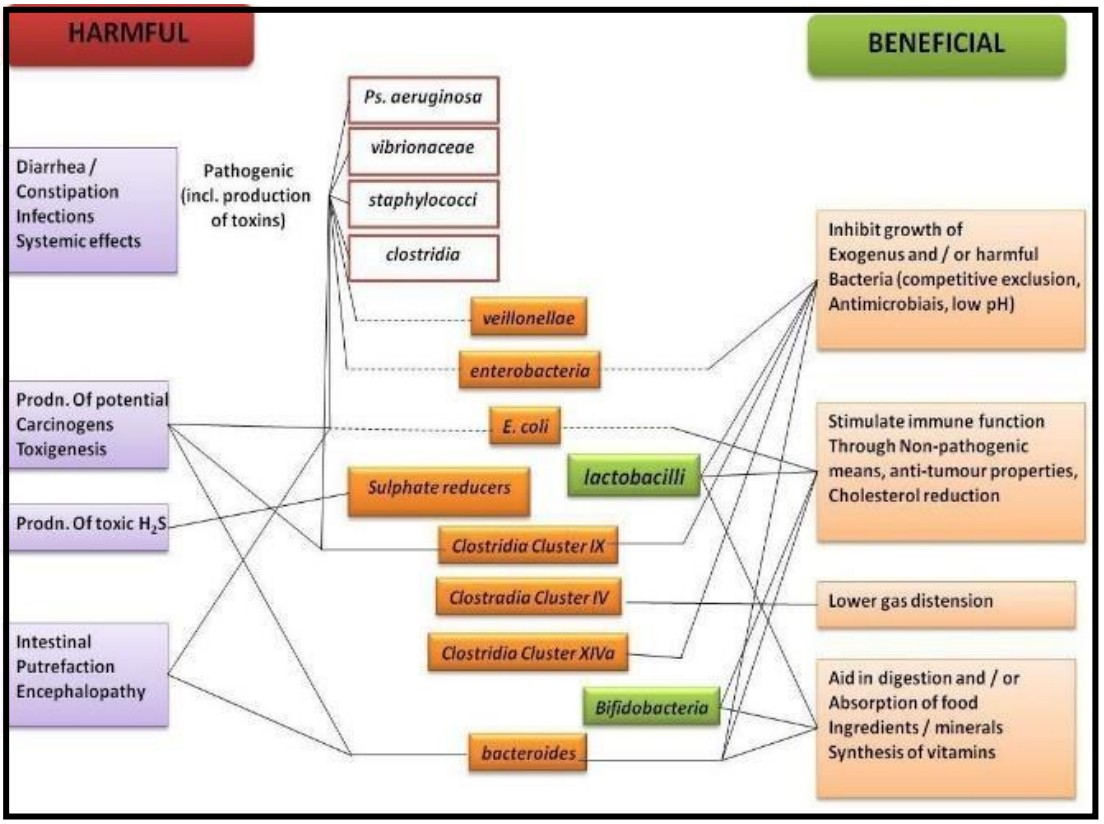

Figure 2.23: Microbial diversity (good and bad) in human gut (Tojo *et al.*, 2014)

2.7.2 Shaping of human gut through life span

The human gut is sterile until birth (Putignani, 2012). Perinatal factors like mode of delivery, feeding regime, gestational age at birth contribute to the initial colonization of the infant gut, (Hallab et al., 2013). It has been shown that vaginally born babies have higher numbers of *Lactobacillus* and *Bifidobacterium*, compared with infants delivered by Caesarean-section (Dominguez et al., 2010).

By the age of one year, babies' distinct bacterial profiles have converged towards the profile of an adult human (Palmer et al., 2007). Gut dysbiosis in the perinatal and neonatal period can lead to the development of a variety of diseases and nutritional deficiencies later in life. The initial colonization is crucial in determining the microflora composition of the adult. Until old age, this remains relatively stable (Clemente et al., 2012). With life events such as bacterial infections, antibiotic therapy, lifestyle, surgery, and a long-term change in diet, the intestinal microbiota undergoes a big shift (Arrieta et al., 2014). Figure 2.24 shows stages of microbial colonization of the infant and child intestine (Arrieta et al., 2014).

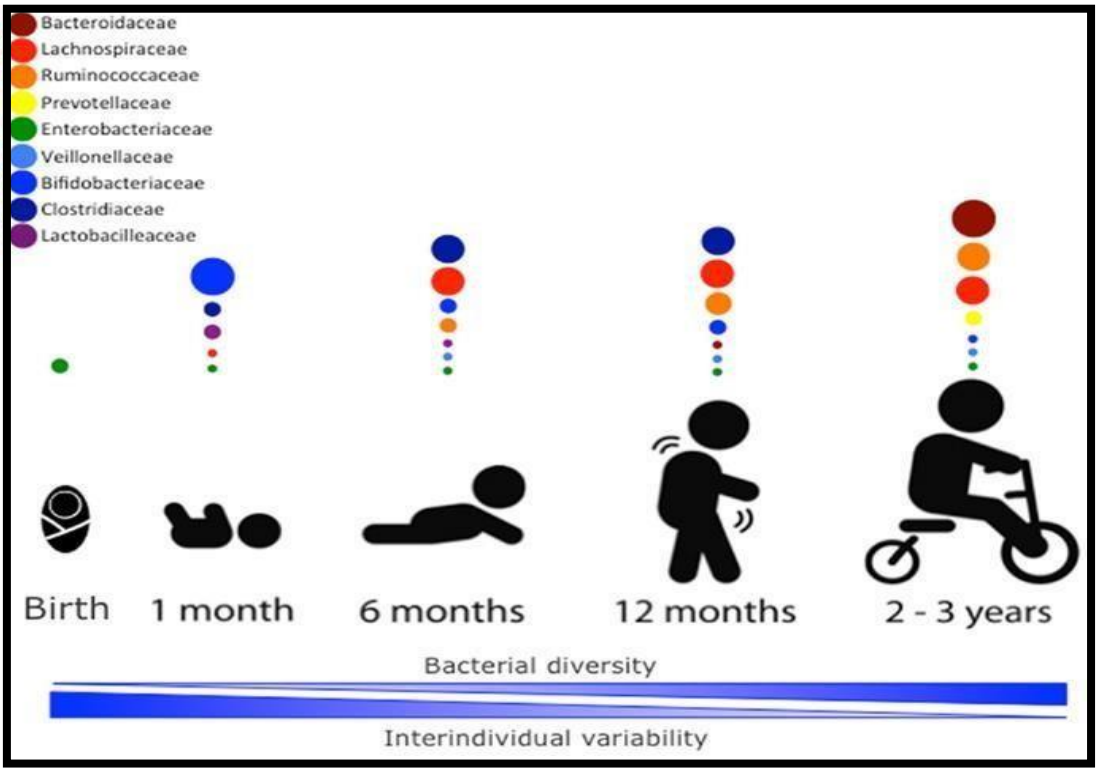

Figure 2.24: Stages of microbial colonization of the infant and child intestine

Microbiota plays major role in maintaining the functionality of intestinal barrier. Maintaining the integrity of barriers is critical for human mental health (Kelly et al., 2015).

2.8 Leaky gut leads to brain functional disorientation

Gut microbiota- Intestinal barrier- Blood Brain Barrier- Brain disorientation

Multifaceted intestinal barrier maintains homeostasis by monitoring nutrient and electrolyte absorption and averting the penetration of uncontrolled luminal content like pathogens and toxic substances into the circulation (Farhadi et al., 2003).

This efficient barrier is preserved by physical, biochemical, and immunological components. the seven intestinal epithelial cells (IECs) enterocytes, goblet cells, paneth cells, microfold cells (M cells), enteroendocrine cells, cup cells, and tuft cells are distributed across the surface area of 400 m2 (Peterson and Artis, 2014).

Enterocytes accounts for at least 90% of crypt cells or villus cells the contact between IECs is sealed by 40 TJ proteins which includes occludin, claudins, junctional adhesion molecule A, and tricellulin (Braniste et al., 2014). Highly glycosylated mucin containing immunoglobulin (Ig) A and antimicrobial peptides lines the epithelium. This intestinal barricade takes it full form by eight week of gestation, epithelial cells with microvilli and enteroendocrine cells and tight junctions are detected from week ten (Louis and Lin, 2009).

Antimicrobial proteins (AMPs) within the mucus layers controls the colonization of commensal bacteria. Reciprocally, the production of some AMPs is regulated by microbiota and their metabolites. Immunoglobulin in intestinal mucosa presumably interacts with commensal bacteria to provide protection against pathogens (Woof and Russell, 2011). Additionally, Gut microbiota provide the energy source to the epithelial cells by the production of SCFA (Krajmalnik et al., 2012). Administration of pre and probiotic restores the property of AMP necessary for physically separating commensal bacteria from intestinal epithelium and control bacterial overgrowth (Yan, 2007).

Homeostasis of central nervous system is maintained by BBB. Its functions are

preserved by endothelial cells which separates the lumen of blood vessels from the CNS parenchyma. Astrocytes and pericytes forms the tight junctions, transmembrane made of protein claudins, tricellulin, and occludin seals the capillary of endothelial cells lining the BBB which prevents diffusion of unwanted substances from blood to the brain (Hawkins and Davis, 2005; Daneman and Rescigno, 2009).

Evidences support the cardinal function of the gut microbiota in the development and maintenance of the intestinal barrier across the lifespan (Ohland and Macnaughton, 2010; Swanson et al., 2011; Shifrin et al., 2012). Dysbiosis of the gut microbiota and its metabolites downregulates the expression of TJs, (Hu et al., 2016) hampering this protective shield, which pose threat to the gut barrier (Camilleri et al., 2012; Bonfrate et al., 2013; Scaldaferri et al., 2013) this syndrome is called leaky gut. Disruption of mucus layers would allow for bacteria penetration, initiating inflammation which leads to diseases (Leclercq et al., 2012; Johansson et al., 2015; Slyepchenko et al., 2016). When bacteria or its metabolites breach the system, they have unrestricted access to the peripheral circulation. The inflammation caused by this microbial translocation forms the link between gut microbiota and neurological diseases including depression (Slyepchenko et al., 2016). This chain reaction cause pathogen to gains entry into the blood stream by acting through type IV pili on bacterial surfaces. Pathogens interacts with molecules on endothelial cells which disrupt the tight packing and pass into the meninges of the brain damaging the BBB integrity. This induces neuron apoptosis, microglia dysfunction, and neurodegeneration, finally causing memory decline, abnormal behavior, and dyskinesia, which are omens of many mental disorders and brain diseases (Daulatzai 2014; Castanon et al., 2015; Sampson et al., 2016).

Cell wall of gram-negative bacteria is composed of endotoxin Lipopolysaccharide (LPS). Release of LPS stimulate macrophages and epithelial cells translocating its way into the bloodstream through increased permeability of tight junctions caused by leaky gut. Healthy microbiota prompts immunocytes to release moderate anti-inflammatory cytokines, such as IL- 10, transforming growth factor beta (TGF-β), and TGF-α, and moderate pro-inflammatory cytokines, such as IL-1β, IL-17, IFN-γ, and tumor necrosis factor alpha (TNF-α), facilitating the appropriate immune responses (Rook and Lowry, 2008; Rothhammer et al., 2018).On contrary,

pathogenic bacteria populating the gut releases endotoxin, modulating signalling pathway, creating an imbalance by triggering the release of various pro-inflammatory cytokines, leading to chronic inflammation which ultimately affects neuroinflammation (de JR et al., 2018). Cytokines adversely influence neurogenesis and neuroplasticity by decreasing brain-derived neurotrophic factor (BDNF) which regulates neurons and synapses involved in emotions and cognition. (Waubant, 2006). 70−90% of depressive individuals report intestinal inflammation, indicating a potential link between the human gut microbiota and cognitive function. Patients with major depressive disorder display differences in the relative abundance of Firmicutes, Bacteroidetes, and Actinobacteria when compared with healthy individuals. infected mice displayed memory dysfunction, reduction in hippocampal BDNF expression and an increase in colonic proinflammatory cytokine levels when exposed to acute stress. (Gareau et al., 2011). Concluding that dysbiosis may have a causal role in the development of depression (Larroya et al., 2018).

Alzheimer's disease, brain trauma, edema, brain cancers (Hu, Y et al., 2021) were observed with breakdown in the blood brain barrier. Recolonization with Bifidobacteria species altered mRNA expression of GABA receptors and decreased serum cortisol and stress response in germ free mice. This change was not seen after the mice underwent vagotomies, suggesting that the parasympathetic nervous system was imperative for the bacteria's effects on their stress response (Cryan and Dinan, 2012). Another study showed alteration of the GF mice gut microbiota with good bacteria decreased the BBB permeability (Braniste et al., 2014).

Gut microbiota is important to healthy brain development and is thought to communicate directly and indirectly with the brain through Gut-Brain axis (Collins et al., 2012).

2.9 Gut Brain axis

The central organ of the nervous system, our brain contains billions of neurons. But we cannot deny the fact that trillions of good bacteria are alive in our intestinal tracts, which makes it far beyond just the organ for food processing and can actually define our mental state. So enteric nervous system can be renamed as

second brain (Spencer et al., 2018). 10^{14} bacteria which make up the intestinal microbiome are engaged in complex interactions with each other and the local tissue, maintaining homeostasis. There is a circular communication loops amid the brain, and gut microbiome. The gut-brain axis consists of bidirectional communication between the central and the enteric nervous system through parallel and interacting channels involving nervous, endocrine, and immune signaling mechanisms, linking emotional and cognitive centers of the brain with peripheral intestinal functions (Carabotti et al., 2015). Healthy intestinal microbiota has the ability to positively regulate the neuroimmune responses in the CNS. Abnormal microbial metabolites may influence neurodegeneration by amyloid formation enhancing inflammatory responses to endogenous neuronal amyloids (Friedland and Chapman, 2017; Hoffman et al., 2017; Minter et al., 2017).

The brain possesses the power to alter the community function and structure of the gut microbiota by modulating regional gut motility through the autonomic nervous system. This affects intestinal transit and secretion, potentially through the luminal secretion of hormones that directly modulate microbial gene expression. Use of short stressors impact the microbiota, exposure to social stressor for only 2 hours significantly was able to change the community profile and to reduce the relative proportions of the main microbiota phyla (Galley et al., 2014). This biological model posits circular communication loops amid the brain, gut, and gut microbiome, perturbation at any level can propagate dysregulation throughout the circuit. This results in altered brain or gut functioning suggesting the existence of a microbiota gut–brain axis (Liang et al., 2018).

The influences of microbiota overstep the gut and reach the whole body, especially the brain, through the microbiota–gut–brain axis (Kelly et al., 2017; Cox and Weiner, 2018; Dinan et al., 2019). Sudo in 2004 first showed that the absence of normal gut microbiota early in life has significant effects on stress responsiveness in the adult and that these changes can be partially reversed by early colonization of the gut with conventional microbiota, even a single species.

Medium of crosstalk between microbiota–gut–brain axis includes

2.9.1 Vagus Nerve

The 10th cranial nerve vagus runs from the brain followed by face and thorax ending in the gut making it longest and the most complex nerve. Studies have revealed that this neural circuit between gut cells and brain stem can transmit signals within mere seconds. To examine if direct neural circuit exist between gut and brain. Neuroscientist investigated cellular connection in mice. A modified, fluorescently-tagged rabies virus was inserted in the colon of mice. Rabies spreads through the body via neurons and reaches the brain, remarkably fluorescent signal was traced as the virus travelled from intestinal enteroendocrine cell all the way through vagal neurons to the brainstem, indicating the presence of a direct line of communication between the gut and the brain (Kaelberer et al., 2018). Figure 2.25 shows overview over the basic anatomy and functions of the vagus nerve (Breit et al., 2018).

Amazing results were revealed when the gut cells from mice were grown up in petri plates in the presence of vagal neurons. the neurons' extensions crawl along the bottom of the dish to connect to the enteroendocrine cells forming synaptic connections with each other. The communication speed up (within 100 milliseconds) on adding sugar solution to the dish. When the same solution was added up to vagal neurons in the absence of gut cells no signal was noticed. This proved that vagus nerve signals can travel faster from gut to brain than hormones (Kaelberer et al., 2018).

There is preliminary evidence that activity of the vagus nerve is also modulated by the gut bacteria (Forsythe and Kunze, 2013; Quigley, 2017; Bonaz et al., 2018). Low vagal tone is prominent in patients with irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Stress induction has also shown to inhibit the vagus neural circuits which has detrimental effect on the gut microbiome. Association between vagal tone and stress biomarker on 26 IBS patients revealed reduced vagal tone and cortisol compared to healthy subjects (Pellissier, 2014). Study performed on 73 subjects with irritable bowel syndrome (IBS) or Crohn's disease showed that subjects had higher score of anxiety and depression symptomatology compared to control. data also argued for an imbalance between

the hypothalamus-pituitary-adrenal axis and the vagal tone in CD and IBS patients Breit et al., 2018). Treatments targeting the vagus nerve emphasize on increasing vagal tone by inhibiting the production of cytokines. Vagal tone is corelated by the activity of gut microbiome with capacity to regulate stress, anxiety and depression like behaviour. Mice when supplemented with probiotics *L. rhamnosus* induced alterations in GABA and mRNA. Concomitant reductions in expression of hippocampus, amygdala, and locus coeruleus was reported. significant reduction in the corticosterone and anxiety- and depression-related behavior was seen. However, the effect of probiotic was eliminated on vagotomy (Bravo, 2011). Other animal studies in which probiotics *Lactobacillus rhamnosus* and *Bifidobacterium longum* were supplemented for suppression of anxiety and depression like behaviors also reflected the same results when the vagus nerve was removed (Forsythe et al., 2013). In addition, the electrophysiological effects of prebiotics stimulating the production of beneficial bacteria which in line affects mental behaviour were also blocked following vagotomy (Vazquez et al., 2016). Targeting the VN, through stimulation dampen peripheral inflammation, is of interest to restore homeostasis in the microbiota-gut-brain axis.

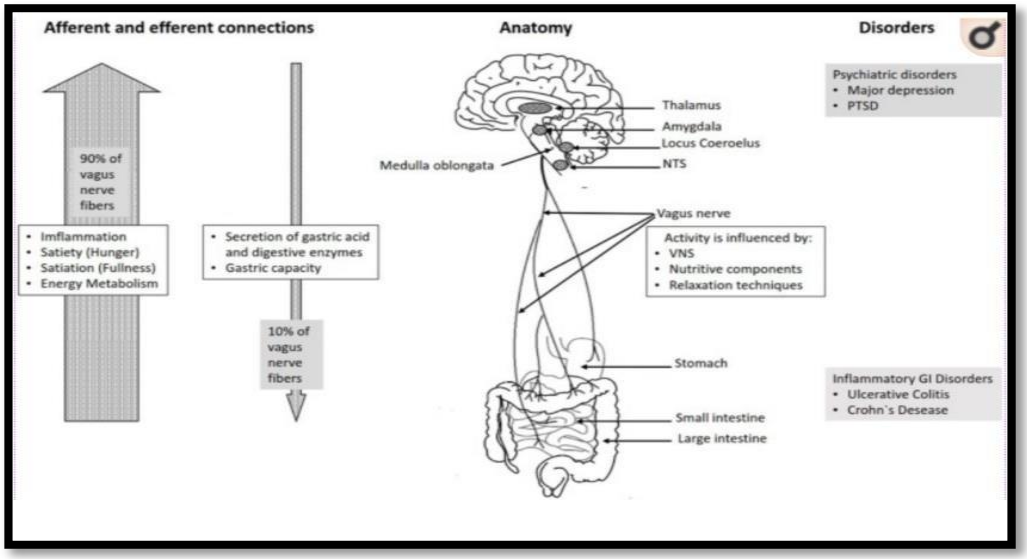

Figure 2.25: Overview of the basic anatomy and functions of the vagus nerve

2.9.2 Neuroendocrine: Hypothalamic–Pituitary–Adrenal axis

The microbial ecosystem is essential to the development and function of the hypothalamic– pituitary–adrenal axis (Liang et al., 2018; Sudo, 2014). This axis

serves as main regulator of homeostasis through hormones forming the basis of major neuroendocrine system. This directs body's metabolism, development, reproduction and reaction to stress.

Maladaptation of gut microbiota induces abnormality in development of the HPA axis. Colonization with beneficial microbes within a critical window prompts HPA axis maturation (Sudo et al., 2004; Liang et al., 2018; Keightley et al., 2015). The exaggerated HPA response by GF mice was reversed by reconstitution with *Bifidobacterium infantis*, but it was exacerbated by mono-colonization with enteropathogenic *Escherichia coli* (Sudo et al., 2014). Probiotic intervention improves the function of the HPA axis both in juveniles and adults (Eutamene and Bueno, 2007; Gareau et al., 2007; Liang et al., 2018; Wang et al., 2015). Chronic stress has also shown to impair the functioning of the HPA axis, even when gut microbiota remains intact.

2.9.3 Neuropeptides

Enteroendocrine cells (EECs) lining the crypts and villi of entire GI mucosa forms the basis for the largest endocrine system in humans (Janssen and Depoortere, 2013). EEC helps in maintaining normal homeostasis by modulating GI functions by release of certain neuropeptides like peptide YY, neuropeptide Y (NPY), cholecystokinin, glucagon-like peptide-1 and -2, and substance P (Greiner and Bäckhed, 2016). There secretion regulates microbiota and their bioactive metabolites which serves as messengers of the microbiota–gut– brain axis (Lach et al., 2018). EEC acts on receptors of vagal afferent fibers which runs from the gut to brain (Bonaz et al., 2018).

Neuropeptide Y (NPY) Family is layered at all levels of gut brain axis outspreading from enteric nerve plexuses and postganglionic sympathetic neurons to cerebral cortex. NPY signaling tunes neuroprotection, neurogenesis and neuroinflammation. Microbial composition is also modulated by them additionally regulating homeostasis, mood, and stress resilience (Holzer and Farzi, 2014; Lach et al., 2018). NPY regulates mainly by altering gastrointestinal activity and immunity. In turn, the microbiota, impacts their synthesis and secretion, and ultimately influences the brain and behavior (Holzer and Farzi, 2014).Thus, it is likely there is abundance evidence pointing that gut microbiota contributes

indirectly to NPY release and signaling to affect its homeostatic role in balancing the brain's response to stress in the brain. Evidence points toward increased colonic synthesis of NPY with depression and anxiety. When gut microbiota was depleted from mice NPY expression within the amygdala and hypothalamus were disturbed, followed by cognitive deficits (Fröhlich et al., 2016).

GLP-1 receptor is secreted critically for neural and hormonal response. These are distributed abundantly in both gut and brain regions (Dickson et al., 2012). GLP-1 target offers beneficial effects in psychiatric disorders. It has involvement in neuroprotection as it helps in reduction of the proinflammatory cytokines. Activation of glucagon-like peptide-1 receptor promotes neuroprotection in experimental autoimmune encephalomyelitis by reducing neuroinflammatory responses (Lee et al.,2018). Diabetic mice showed suppression in anxiety on treatment with GLP-1 receptor agonist (Komsuoglu et al., 2014). Ability of this receptor in reversing LPS-induced depressive-like behavior has also been proved (Ventorp et al., 2017). GLP-1 treatment can reverse the mood disorders and cognitive deficits (McIntyre et al., 2013).

Ghrelin, approximately 75- 80% of circulating ghrelin is secreted by Gastrointestinal tract. Primary function of this gravimetric system is to act as hunger signal (Steiger et al., 2011). Recent researches have pointed that via active transport and direct diffusion ghrelin can penetrate Blood Brain Barrier (BBB) and bind the hippocampus. which is indicative of gut brain connection of this neuropeptide. Rodents when induced with stressors have shown increase in long-term ghrelin levels of the stomach. (Micael et al., 2008) found that subcutaneous injections to increase ghrelin levels in mice produced anxiolytic and antidepressant responses in the elevated plus maze and forced swim test. The impact of ghrelin on psychopathology, sleep and secretion of cortisol and GH in patients with major depression was studied by (Kluge et al., 2011). An improvement at trend level in men (placebo: 36 ± 9 to ghrelin: 30 ± 9, $p = 0.093$) was noticed. Ghrelin was associated with less time awake (placebo:149.0 ± 11.1; ghrelin: 88.0 ± 12.2 min, $p = 0.029$) and more non-REM sleep (placebo: 263.2 ± 24.1; ghrelin: 304.9 ± 14.1 min, $p = 0.027$). Concluding initial indication that ghrelin can exert antidepressant effects in patients with major depression. Targeting the release of the gut peptides may be an approach to be considered in conjunction with future

breakthroughs in psychobiotics.

2.9.4 Short Chain Fatty Acid

SCFA are small organic monocarboxylic acids with a chain length of up to six carbons atoms. Approximately 500–600 mmol of SCFAs are produced in the gut per day and it influence gut- brain communication directly or indirectly (Macfarlane and Macfarlane 2003).

Acetate, propionate, and butyrate in an approximate molar rate of 60:20:20, respectively are the main metabolites produced by the microbiota in the large intestine (Pascale et al., 2018). Other SCFAs, namely, formate, valerate and caproate, are produced in lesser amounts. Following their production, SCFAs are absorbed by colonocytes, mainly via H+-dependent monocarboxylate transporters (MCTs) or sodium-dependent monocarboxylate transporters (SMCTs) (Wall et al., 2014; Koh et al., 2016). They interact with their receptors present on enteroendocrine cells which promotes indirect signaling to the brain via the systemic circulation or vagal pathways by inducing the secretion of gut hormones such as glucagon-like peptide 1 (GLP1) and peptide YY (PYY), as well as γ-aminobutyric acid (GABA), and serotonin (5-HT).

Peripherally, SCFAs influence systemic inflammation mainly by inducing T regulatory cells (Treg) differentiation and by regulating the secretion of interleukins. SCFAs can cross the blood-brain barrier (BBB) via monocarboxylate transporters located on endothelial cells and influence BBB integrity by upregulating the expression of tight junction proteins (Erny et al., 2015).Finally, in the central nervous system (CNS) SCFAs also influence neuroinflammation by affecting glial cell morphology as well as by modulating the levels of neurotrophic factors which increases neurogenesis, contributing to the biosynthesis of serotonin, and improving neuronal homeostasis and function thereby potentially affecting emotion, cognition and pathophysiology of mental disorders. In line with these findings, clinical evidence has shown that fecal SCFA concentrations are lower in patients with depression than in controls (Skonieczna et al., 2018). Sodium butyrate has been shown to be capable of reversing behavioural hyperactivity and depressive-like and manic-like behaviours in rats (Resende et al., 2013). There is

also evidence for butyrate's antimanic effect on a rat model of bipolar disorder induced by intracerebroventricular administration of ouabain (Valvassori et al., 2017). Systemic injection of butyrate induced histone hyperacetylation in the hippocampus and frontal cortex and exerted antidepressant like effects in mice that were associated with increased BDNF transcripts in the frontal cortex. Propionic acid has also been suggested to have significant effects since intraventricular infusions of propionic acid induce irreversible behavioral changes which have been likened to those seen in autism (MacFabe et al., 2007).

2.9.5 Neurotransmitters

These are endogenous chemicals which enables signals transition between neurons forming a chemical synapse (Lodish et al., 2000). Gut microbiota has potential to produce a range of major neurotransmitters directly (Wall et al., 2014; Mazzoli and Pessione, 2016). More than 90% of body's serotonin and 50% of dopamine (DA) are synthesized in the gut (Sudo 2014; Yano et al., 2015). Strains of *Bacillus* and lactic acid bacteria (LAB) species, have shown to synthesize catecholamines and acetylcholine (Wall et al., 2014). Study by Mazzoli and Pessione in 2016 showed that several LAB strains are also able to produce glutamate. *Candida*, *Streptococcus*, *Escherichia*, and *Enterococcus were able to* synthesize 5-HT (Holzer and Farzi, 2014).

Dopamine and Norepinephrine

Dopamine is the main chemical of pleasure and the component of reward-motivated behavior (Baliki et al., 2013). It is also the precursor for catecholamines, like norepinephrine and epinephrine, which are known for their role in arousal and alertness in the waking state, sensory signal detection, behavior and cognition, like memory, learning, and attention (Borodovitsyna et al., 2017).

Interestingly, researches have shown that bacteria respond to or produce these catecholamines stating the connection between brain and gut. *Escherichia coli* O157:H7 (EHEC) showed increase in growth rate in the presence of dopamine and norepinephrine (Freestone et al., 2007). *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Shigella sonnei*, and *Staphylococcus aureus* were all found to have improved growth *in vitro* in the presence of norepinephrine (O'Donnell et al., 2006). A study leveraging germ free mice found substantially

reduced levels of norepinephrine in the cecal lumen (35 ± 5ng/g compared to 3.8 ± 1.3 ng/g) and in the tissue (115 ± 14 ng/g vs. 5.0 ± 0.5 ng/g), levels of norepinephrine were restored via colonization with a mixture of 46 *Clostridia* species (Asano et al., 2012).

Serotonin and Melatonin

Serotonin popularly called the body's natural feel-good chemical is responsible for our mood and happiness factor. Pharmaceuticals involved in treating depression, anxiety, and other mood disorder targets serotonin. Evidences has shown gut bacteria influences serotonin levels in the body by playing a major role in intestinal mobility (Jenkins et al., 2016).

More than 50% of serotonin was found missing in the gut of mice without the bacteria. When bacterial mixture of Turicibacter sanguinis and Clostridia, was introduced, mice produced molecules that signalled the gut cells to increase serotonin production. In another research one group was introduced to serotonin added drinking water while other group of mice was raised with mutation (created by altering a specific serotonin transporter gene). Increased level of serotonin with Increase in population of bacteria Turicibacter and Clostridia was reported (Fung et al., 2019). Wikoff in 2009 compared the levels of serotonin in blood and colon of normal vs. germ free mice. Significant difference was seen between the two groups. Production of Serotonin is mediated by bacteria signal which further regulates the melatonin secretion. proving that gut bacteria signaling is sensitive to melatonin production. Neurohormone melatonin regulates circadian rhythm which further determines our mood (Paulose et al., 2016; Thaiss et al., 2017).

The balance of gut microbiota is vital for maintaining the brain concentration of 5-HT, by the inhibition of the kynurenine. This change in the pathway ultimately decreases serotonin production, which plays a key role in depression. Stress causes a switch in the tryptophan metabolism pathway to kynurenine. Tryptophan, an integral amino acid, is converted to serotonin in normal circumstances (5-HT). Tryptophan is degraded to the kynurenine pathway by stress-induced gut inflammation, which is triggered by the release of cytokines (IL-1,IL-6, TNF). Kynurenine is converted to kynurenic acid by the enzyme kynurenine aminotransferase I,II,III, and to 3 hydroxy kynurenine by the enzyme kynurenine

hydroxylase. It is further broken down to end metabolites quinolinic acid and picolinic acid. NAD, the end metabolite of quinolinic acid has neurotoxic effects which acts as NMDA receptor agonist, having role in involvement of several psychiatric disorders including hydroxykynurenine, mood disorders and depression (Bosi et al., 2020). Hyper stimulation of kynurenine pathway, makes brain to cut off protein pump which would otherwise allow serotonin to enter and circulate within brain cells (Liang et al., 2018).

Gamma-aminobutyric acid (GABA)

This neurotransmitter induces natural calming effect. Altered levels of GABA leads to numerous CNS disorders, including behavioural disorders, pain, and sleep (Wong et al., 2004). It also plays vital role in maintaining the functionality of Enteral Nervous System such as intestinal motility, gastric emptying, nociception, and acid secretion (Hyland and Cryan, 2010) determining its association with both gut and brain.

Researches have proved that biosynthesis of GABA is dependent on gut bacterium. Luminal and serum levels of GABA were substantially reduced in germ-free mice (Matsumoto et al., 2013). Introduction of good gut bacteria specifically of the species Lactic Acid bacteria and *Bifidobacterium* have been reported to produce GABA. Supplementing *Lactobacillus rhamnosu*s reduced depressive- and anxiety-like behavior accompanying changes in cerebral GABAergic activity (Bravo et al., 2011). Reduction in sensitivity to visceral pain was noticed in a rat model when oral supplemented with *Bifidobacterium breve strain* engineered to produce GABA, (Pokusaeva et al., 2017). Human fecal sample bioassay-guided fractionation of *B. fragilis* supernatant showed surprising dependency upon gamma-aminobutyric acid (GABA) for in vitro growth of KLE1738 that required *Bacteroides fragilis* for growth (Strandwitz et al., 2019).

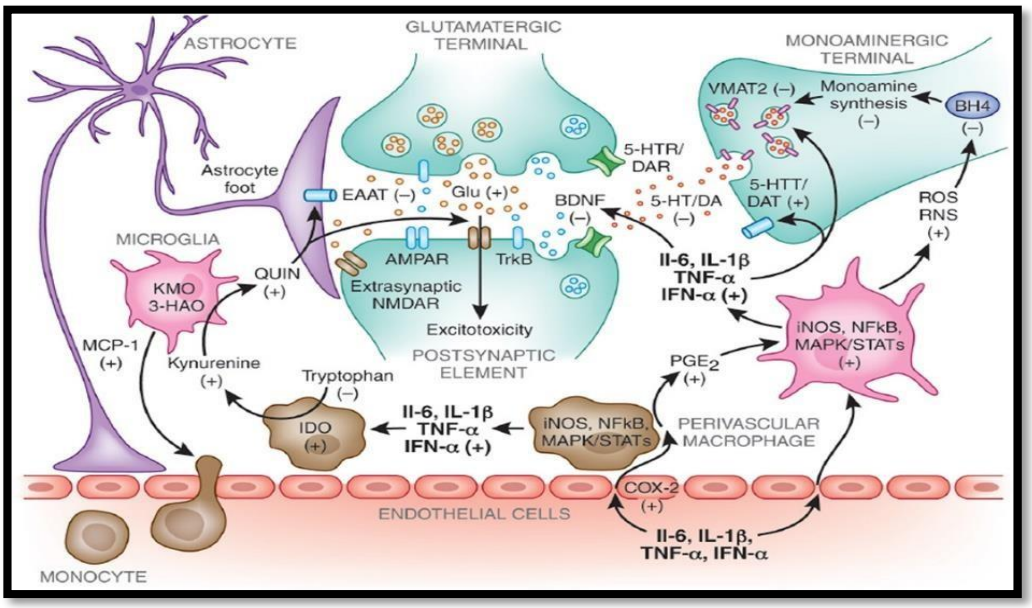

Figure 2.26: Potential mechanism of inflammatory cytokine effects on brain monoamine, glutamate and BDNF neurotransmitter

We are aware that gut microbiota is the main link between all possible linkage of gut and brain chain. to Promote Brain and Mental Health Microbiota modulation is the key.

2.10 Proven strategies for microbial modulation

Human gut microbiota can be modulated by various techniques.

2.10.1 Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is the process in which healthy colon bacterium can be transferred or shared by transplanting feces from a healthy donor to the receiver gut possessing the weak colonies (Aroniadis and Brandt, 2013).

FMT has proven out to be effective treatment against inflammatory bowel disease and digestive problems (Zhang et al., 2018). Evidences have also pointed FMT as effective therapy for various mental disorders like depression (Evrensel and Ceylan, 2015), Tourette Syndrome (Zhao et al., 2017), and epilepsy (He et al., 2017). Fecal Microbiota Transplantation of mild stressed mice to normal mice induced anxiety and depressive behavior in recipient (Li et al., 2019). Similar results were seen in human study. Improvement in mental health and decreased frequency of irregular bowel was noticed in patients (45) with recurrent C.

difficile infection, when treated with FMT over the other group which had antibiotic treatment (Jalanka et al., 2018).

This microbial target therapy has evolved into frozen (FMT) capsules that can be taken orally, and is no longer limited to colonoscopy. Clinical trial (NCT0328104) was launched in 2018 at University Psychiatric Clinics (UPK), Switzerland to determine this adjuvant therapy's function in counteracting depression claims include improvement in hypothalamic-pituitary-adrenal (HPA) axis responses, neurogenesis, energy balance hormones, gut microbiota composition, and brain perfusion, structure, and activation. FMT has proved out to be an excellent technology for colonizing the gut with good microbes thereby, improving the gut brain axis and associated metabolic disorders. But the potential cost of transfer of a gut microbiota profile may increase vulnerability to another gut microbiota contributory disease (Mullish, 2018).

2.10.2.1 Probiotics

The concept and property of probiotics gained its importance long before this term was introduced. Over a century ago, in 1907 Elie Metchnikoff presented the rationale for intestinal auto-intoxication caused by putrefactive bacteria and associated it with ageing. The scientist thereafter experienced health benefit of consuming fermented milk himself. The probiotic concept introduced by Metchnikoff led to the development of the first dairy industry in Europe (Mackowiak, 2013). Another scientist from Pasteur Institute Henry Tissier gave remarkable contribution by isolating *Bifidobacterium* and claiming it as the treatment for diarrhoea in babies (Tojo, et al., 2014).

Werner Kollath, a German scientist, coined the word probiotic in 1953 to describe "active substances necessary for life's healthy growth." (Kollath, 1953). In 1965, Lilly and Stillwell coined the term "probiotic," which they described as "substances secreted by one organism that stimulate the growth of another" (Lilly, 1965).

It was in 2001 when FAO/WHO jointly defined Probiotics as **"Living microorganisms which when administered in adequate amount conferring health benefits of the host" or microbially** derived factors that stimulate growth

of other microorganisms (Morelli and Lorenzo, 2012). Probiotics have shown their efficacy not only in the disorders associated with GI tract, but their role have been well established in maintaining immune function, reducing the risk of metabolic disorders like diabetes, obesity, cancer, oral health, acute respiratory tract infections as well as in neurodegenerative diseases (Amara and Shibl, 2015).

The consumption of a daily probiotic by healthy children resulted in a 25% reduction in the number of days missed at school, according to day care center studies (Weizman, 2015).

The promising role of probiotic in disease associated with intestine can't be denied. Probiotics work by maintaining the integrity of the gut barrier, Inhibition of bacterial adhesion, improved mucosal barrier function, control of the innate and adaptive immune systems (including activation of tolerogenic dendritic cells and regulatory T cells), secretion of bioactive metabolites; and regulation of the enteric and central nervous systems are among the other mechanisms of action (Dudek et al., 2020).

Enterotoxigenic *E. coli* adhesion to porcine enterocytes and diarrheagenic *E. coli* adhesion to human intestinal epithelium have also been shown to be inhibited by *Lactobacillus strains* (Tran et al., 2018). L. plantarum has been shown to improve intestinal barrier function by increasing mucin (MUC2 and MUC3) production and secretion from human intestinal epithelial cells (Dykstra et al., 2011). Strains of *Lactobacillus* and *Bifidobacterium* produce cytoprotective molecules, which *stimulate epithelial cell signalling pathway.* Feeding *Lactobacillus rhamnosus* to new born mice reduced intestinal injury and inflammation by enhancing epithelial cell proliferation, differentiation, tight junction formation, and mucosal IgA production (Yuan et al., 2017). Individuals with IBS respond to probiotics with a reduction in symptoms, such as flatulence, abdominal pain, and constipation. Children with functional abdominal pain benefited from probiotics *S. cerevisiae CNCM* I-3856 and *B. infantis* 35624. *L. reuteri* as a reduction in the frequency and intensity of pain episodes was seen (Weizman et al., 2016). The seven core genera of microbial organisms most often used in probiotic products are *Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus, Escherichia,* and *Bacillus* (Probiotic factsheet 2020, NIH).

Probiotic cocktail made up of *Lactobacillus* and *Enterococcus* when inoculated in the human feces ameliorated gut microbiome dysbiosis and increased production of SCFA. When the dosage of same cocktail was given to mice similar effect was observed. SCFA which particularly showed increment were propionate and butyrate (Nagpal et al., 2018). Figure 2. 27 illustrates diverse mechanisms likely to drive probiotic benefits to host health (Balcazar, 2007)

The principle of bidirectional brain-gut-microbiome (BGM) interactions supports the role of probiotics in improving various psychiatric conditions in preclinical studies. The ability of probiotics and prebiotics to produce and deliver neuroactive substances such as gamma- aminobutyric acid and serotonin (the "happy" chemical), while decreasing cortisol (the "stress" hormone) and increasing oxytocin (the "cuddle" hormone), has proven its role in psychiatric illness, earning them the moniker "psychobiotics." (Dudek et al., 2020).

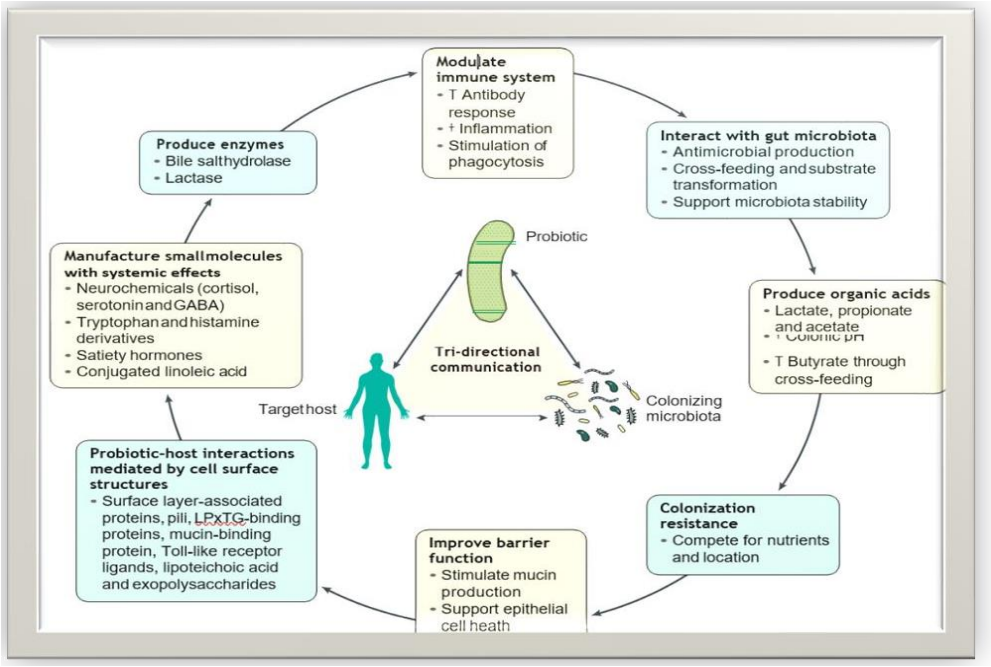

Figure 2.27: Diverse mechanisms are likely to drive probiotic benefits to host health

The administration of probiotic bacteria reduced inflammation, restored epithelial barrier function, and potentially ameliorated behavioural symptoms among children with autism (Critchfield et al., 2011).

Some probiotics are used as important component of certain anti-depressant medicines and acts as effective fertilizers in improving mental disorders like AD,

Parkinson's, Autism and depression (Pirbaglou et al., 2016; Sanchez et al., 2017). Even eating probiotic foods to reduce depression is as effective as antidepressants. Yogurt is made by fermenting milk with *Lactobacillus rhamnosus*, which promotes the release of neurotransmitter GABA well known for reducing anxiety (Yong et al., 2020). Valium and other anti-anxiety drugs also act by focusing on GABA receptors in the brain. Specific Lactobacillus strains are shown to mimic the the effects of morphine in promoting analgesia modulating pain perception (Aziz Q et al., 2013).

The fecal microbiota of Parkinson's patients displayed a reduced abundance of *Prevotellaceae* and elevated levels of *Enterobacteriacea*e compared to healthy control groups (Gerhardt and Mohajeri, 2018). McMaster University in Ontario published a study in based on their findings that mice given the bacterium Lactobacillus rhamnosus were more relaxed than other mice during the experiment (Neufeld, 2011). Human trials yielded similar results. Gamma aminobutyric acid (GABA), a major inhibitory neurotransmitter in the human central nervous system (CNS), was increased by P. Lactobacillus brevis and Bifidobacterium dentium (Barrett et al., 2012). Taking probiotics containing Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 for 30 days reduced psychological stress, particularly depression, anger-hostility, and anxiety, and improved problem solving (Messaoudi, 2011). A 12-week intervention with Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, and Lactobacillus fermentum improved cognitive function and some metabolic statuses in Alzheimer's disease patients (Akbari et al., 2016). Another study found that patients who received the probiotics *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* (2 billion CFUs each) for eight weeks had significantly lower scores on Beck Depression Inventory (Akkasheh et al., 2016).

2.10.3 Prebiotics

Research in 1950, indicated that babies who were breast fed had rich population of bifidobacterial in their gut. This pointed towards the presence of bifidus factor' in human milk (György et al., 1954). Prebiotics were defined by Glenn and Marcel in 1995 as a "non- digestible food ingredient that benefits the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the

colon, thereby improving host health (Gibson and Roberfroid 1995)." However, at the 6th Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) in 2017, the definition was refined as "A prebiotic is a substrate that is selectively utilized by host microorganisms conferring health benefit." Table 2.4 defines main prebiotic definitions evolved from 1995 until 2017.

Table 2.4: Main prebiotic definitions evolved from 1995 until 2017

Year	Prebiotic Definition	Reference
1995	A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health.	Gibson and Roberfroid 1995
2003	Prebiotics are nondigestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria.	Leenen and Dieleman, 2007
2004	A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health.	Gibson et al., 2004
2010	A prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the	Gibson et al., 2010

Year	Prebiotic Definition	Reference
	gastrointestinal microbiota, thus conferring benefit(s) upon host health.	
2017	A prebiotic is a substrate that is selectively utilized by host microorganisms conferring health benefit.	Gibson et al., 2017

Prebiotic supplements, as opposed to probiotics, serve as food for colonic bacteria that are already present in the gut ecosystem. They play an important role in the metabolism of complex carbohydrates and plant polysaccharides that human enzymes are incapable of digesting completely. As primary end products, this metabolism generates SCFAs such as acetate, propionate, and butyrate. The majority of the prebiotic fermentation product is acidic, which lowers the pH of the gut. It has been demonstrated that a single unit change in gut pH from 6.5 to 5.5 can contribute to a change in the composition of the gut microbiota and promote the formation of butyrate; this is known as the butyrogenic effect (Davani et al., 2019). Butyrate is an important metabolite that affects gut health because it is a primary energy substrate for colonocytes and enterocytes. More importantly, prebiotic supplements allow for the cross-utilization of by-products among bacterial community members, which stimulates the growth of other microbes and thus increases bacterial diversity, which probiotic supplements alone cannot achieve (Holscher, 2017). Fructans, FOS, GOS, and XOS are some popular prebiotics that have been shown to have health benefits and are industrially produced (Davani et al., 2019).

The criteria required for a food ingredient to be termed as prebiotic are: o
Resistant to gastric acidity

- Resistant to hydrolysis by mammalian enzymes
- Resistant to Gastro intestinal tract absorption
- Fermentation by intestinal microflora
- Selectively stimulates the growth and/or activity of intestinal bacteria associated with health and wellbeing (Gibson et al., 2010).

2.10.3.1 FOS as a prebiotic

FOS is an abbreviation for fructose oligomers, which are also known as inulin-type oligosaccharides. They are a class of homologous oligosaccharides with the formula GFn derived from sucrose (Kelly, 2008). FOS is not digested by the human digestive tract, but when it reaches the colon, it stimulates the growth and strength of specific bacteria in the intestine, which is beneficial (Gibson and Roberfroid, 1995). The average counts of bifidobacteria increased, while Bacteroides, Fusobacterium, and Clostridium sp. decreased significantly. ßfructosidase, which is secreted by bifidobacteria, is the enzyme responsible for FOS hydrolysis (Robertfroid, 1993).

2.10.3.2 FOS safety, tolerance and Caloric value

Roberfroid, calculated the caloric value of FOS to be about 1.0 kcal/g to 1.5 kcal/g using a different approach based on lipogenesis equilibrium (Roberfroid, 1993). FOS intake in Holland is estimated to be between 2 and 12 grammes per day. In Japan, the daily dose is estimated to be about 13.7 mg/kg of body weight. However, the Japanese law specified a daily intake of 0.8 g/kg of body weight as an adequate daily intake for FOS approval (Tuohy et al., 2003). Flamm in 2001, calculated the caloric value of FOS and estimated the energy yield for the host to be between 1.5 and 2.0 kcal/g. The average per capita daily consumption of FOS is 2 - 4 g for North Americans and 2 - 12 g for Europeans (Sousa et al., 2011). In Brazil, there are no relevant data regarding the amount consumed or the dietary recommendations. The law considers FOS as ingredients of products, not additives. Ingestion may cause flatulence, especially in individuals who have lactose intolerance, but the severity of this symptom is associated with the amount of FOS consumed (Hauly and Moscatto., 2002). A s per US, FDA notice no. GRN 000118 FOS consumption is regarded as safe (Office of food additive safety). The tolerance and the threshold dose of short chain FOS was studied by Bouhnik et al., 1999.

Healthy volunteers were divided into 5 groups and were treated with oral doses of a powdered mixture of 0 – 20g of short chain FOS. The results concluded that to increase the bifidogenesis without any side effects such as flatulence, was 10g per day. Evidence points in the direction that FOS dose as less than 4gm/ day is also sufficient to bring about colonization of good gut bacteria (Tuohy KM et al., 2003). However, these doses vary greatly between individuals and also depend on the type of food that contains inulin or oligofructose. Increased flatulence and osmotic pressure can cause intestinal discomfort at high doses. The FDA estimates that the 90th percentile dietary intake of inulin type fructans would be approximately 6 g per day for infants under one year of age, approximately 15 g per day for infants one year of age, and approximately 20 g per day for the general population (i.e., two years of age and older).Roberfoid, 1999 recommends that inulin and oligofructose, as well as all nondigestible oligosaccharides that are mostly fermented in the colon, be assigned a caloric value of 1.5 kcal/g (6.3 kJ/g) for nutrition labelling purposes.

2.10.3.3 Sources of FOS

FOS is found naturally in approximately 36,000 plants, including asparagus, beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato, rye, soybean, human and cow milk, peas, beans, seaweeds, and microalgae (Padma and Pichan, 2013). Due to the low concentration in these foods, FOS is synthesized on a large scale.

2.11 Implications OF FOS on Gut and brain

Prebiotics hypothesized to confer health benefits to the host by amplifying the growth of gut microbial species. This mechanism has been well documented, which clears its role in prevention and control of various diseases emphasizing the crosstalk between gut and brain. (Sabater et al., 2009).

Both human and animal data have shown a powerful impact of prebiotic in improving gastrointestinal tract diseases (Guarino et al., 2020). Prebiotics increases saccharolytic activity within the gut and promote the growth of *good bacteria*. Supplementing 15g fructooligosaccharide for 15 days to human volunteers demonstrated marked increase in the number of *Bifidobacterium* (Gibson et al., 1995). Another study

investigated the prebiotic efficacy of biscuit's containing 6.6g FOS, significant increase was seen in the *Bifidobacterium* levels of subjects consuming biscuits daily. Study in Japan revealed, 4g of fructooligosaccharide per day would be needed to observe an increase in the gut *Bifidobacteria* (Tuohy et al., 2003).

Patients with ulcerative colitis and crohn's disease who were exposed to different doses of FOS from 12g/d to 15g/d for 3-4 weeks reported decreased mucosal inflammation in both the gastrointestinal disease conditions (Furrie et al., 2005; Lindsay et al., 2006). Study conducted in 2018 on 83 children with diarrhea demonstrated normalized stool consistency by day 4 on receiving symbiotic daily for 5 days (Houeiss et al., 2018). When oligofructose was introduced to genetically obese mice it increased the levels of *Lactobacillus*, *Bifidobacterium*, and *C. coccoides*, *E. rectale* , which led to a reduction in intestinal permeability and an improvement in tight junction integrity. Inflammatory markers, such as lipopolysaccharides and cytokines were also reduced (Million et al., 2013). Similar results were observed in 65 obese adults who were supplemented 12 gm FOS opposite to placebo group which received 12 g of dextrose for a period of 12 weeks. Improvement in plasma GLP–1 level along with significant increase in *Lactic acid bacteria* and *Bifidobacterium* by 14% and 10% respectively and 20% reduction in enteric pathogen was seen (Sheth et al., 2014).

People who took FOS were less attentive to negative stimuli in an attention dot probe task, according to Phil Burnet and Kristin Schmidt at Oxford in England. The amount of time it took researchers to complete that task provided researchers with an idea of their attention span. Consuming fructooligosaccharide synthesized from Morinda officinalis showed improvement in oxidative stress and inflammation disorder. Secretion of neurotransmitter which improve effective memory were also regulated (Chen D et al., 2017).

A 2018 study looked at the composition of faecal Bifidobacterium after mice were fed FOS. Actinobacteria levels were significantly elevated. demonstrating Bifidobacterium's ability to metabolize FOS (Mao et al., 2018). FOS+GOS treatment had both antidepressant and anxiolytic effects, reducing stress-induced corticosterone release and normalizing the effects of stress on the microbiota (Burokas et al., 2017).

> The processes required for fermented foods were present on earth even before man appeared on the scene… When we study these foods, we are in fact studying the most intimate relationships between man, microbe and foods (Steinkraus et al.,1993).

2.12 Fermentation: an age-old miracle invention

Fermentation can be traced back to 7000 BC (Mc Govern et al., 2004). It is the oldest food technology that humans have used inadvertently. They serve as both preservers and transformers. Fermented foods gained popularity after it was discovered that they have the ability to magnify the known benefits of a wide range of foods by influencing the bioavailability and activity of biogenic metabolites that play critical roles in health (Dimidi et al., 2019).

Dairy is the oldest fermented food, dating back before the invention of alcohol. Back in 10,000 BC, people living in subtropical climates noticed that the milk of camels, goats, sheep, and cattle changed due to naturally occurring microflora in the milk. The first yoghurts were made in goat bags draped over the backs of camels in the heat of North Africa, where temperatures hovered around 110°F during the day, creating ideal conditions for fermentation. In 1856, Louis Pasteur defined fermentation as "respiration without air," implying that it involved some cellular propagation. The role of microbes in the process was later discovered (Dhewa et al., 2015).

2.12.1 Fermentation process

A batch fermentation can be thought of as a closed system. The sterilized nutrient solution in the fermenter is inoculated with microorganisms at time (t=0), and incubation is allowed to continue. During the course of fermentation oxygen (in the case of aerobic microorganisms), an antifoam agent, and an acid or base to control the pH is added. As a consequence of the cells' metabolism, the composition of the culture medium, biomass concentration, and metabolite concentration all change on a regular basis. Four distinct phases of growth are observed after microorganisms are inoculated into a sterile nutrient solution and cultured under physiological conditions as indicated in Figure 2.28 (Paulová et al., 2013).

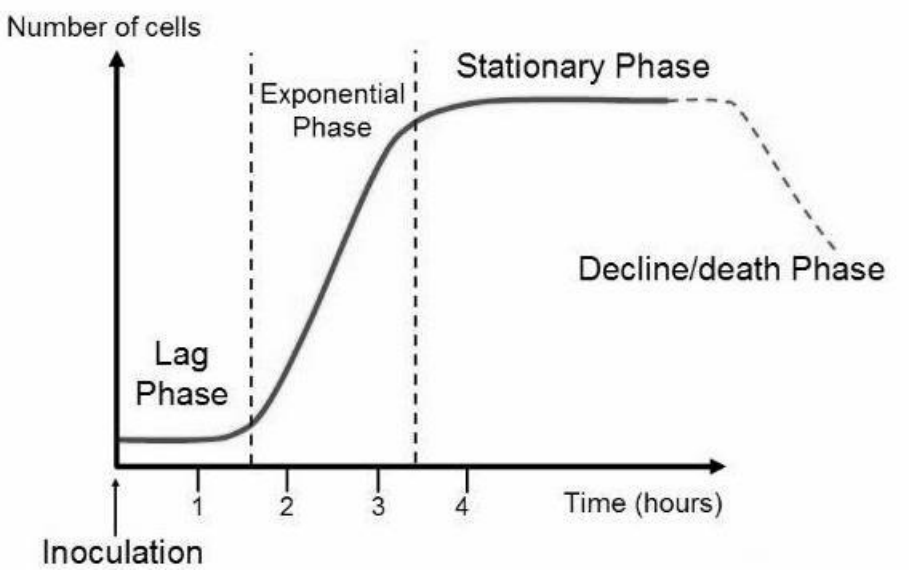

Figure 2.28: Growth curve of a bacterial culture

Lag phase

Physicochemical equilibration between microorganism and the environment following inoculation with very little growth (Paulová et al., 2013).

Log phase

The cells have adapted to the new growth conditions by the end of the lag process. For bacteria and yeast, cell mass growth can now be quantified as a doubling of cell number per unit time, or a doubling of biomass per unit time for filamentous species like fungi. A straight line is obtained by plotting the number of cells or biomass against time on a semilogarithmic graph. As a result, the word log process was coined. During the log step, the cells modify the medium by absorption of substrates and excretion of metabolic products, but the growth rate remains constant. As long as there is excess substrate, growth rate is independent of substrate concentration (Paulová et al., 2013).

Stationary phase

As soon as the substrate is metabolised or toxic compounds are produced, growth slows or stops entirely. During this stationary process, the biomass increases slowly or remains constant, though the cell composition can change. New substrates are released as a result of lysis, which can serve as energy sources for survivors' slow development.

The numerous metabolites generated during the stationary phase are frequently of biotechnological interest (Paulová et al., 2013).

Death phase

The cells' energy reserves are depleted during this process. When a semilogarithmic plot of survivors versus time is made, a straight line can be obtained, suggesting that the cells are dying at an exponential rate. The time it takes for a microorganism to transition from stationary to death depends on the microorganism and the mechanism used. The fermentation process is normally halted near the end of the log phase (Paulová et al., 2013).

2.12.2 Functional properties of fermented foods

According to scientific evidence, many fermented foods contain both nutritive and non- nutritive components that have the ability to modulate specific target functions in the body that are related to the consumers' well-being. However, 90 percent of naturally fermented foods and alcoholic drinks are still produced at home in various countries and regions around the world.

Both functional and non-functional microorganisms can be found in naturally fermented foods and beverages (Tamang et al., 2016). During food fermentation, functional microorganisms transform the chemical constituents of raw materials enhancing nutrient bioavailability, enriching sensory quality of the food, imparting bio-preservative effects and improving food safety, degrading toxic components and anti-nutritive factors, producing antioxidant and antimicrobial compounds (Bourdichon et al., 2012; Tamang, 2015).

Functional properties of microorganisms in fermented foods include probiotics properties (Hill et al., 2014), antimicrobial properties (Meira et al., 2012), antioxidant (Perna et al., 2013), peptide production (De and Dia, 2010), fibrinolytic activity (Kotb, 2012), poly-glutamic acid (Chettri and Tamang, 2014), degradation of antinutritive compounds (Babalola, 2014), etc. Based on their characteristics starter cultures are selected by functional food manufacturers (Badis et al., 2004).

Fermentation was known only for its preservative role before its value as therapeutic came forward. When the culture grows the pH drops down inhibiting

the growth of putrefactive bacteria adding to the product's shelf life (Rakhmanova et al., 2018).

Elie Metchnikoff, a bacteriologist, proposed the role of fermented products in providing health benefits in 1910. He was the first to propose live microorganisms can modify gut microbiota as a cure to improve health. He demonstrated that gut bacteria can be modulated and are affected by the foods we eat by linking the increased longevity of Bulgarians to the regular consumption of fermented milk, which was not practiced in other cultures. He named the bacteria in the fermented milk Bulgarian bacillus, which was later renamed Lactobacillus bulgaricus (Podolsky, 2012). Russian Scientist Ilya Mechnikov in 1905, showed that eating curd is very healthy, since it introduces helpful bacteria like *lactobacillus*, and *bifida*. For this path- breaking discovery, Mechnikov shared the Nobel Prize in 1908. The lactose in the milk transfigures to lactic acid when inoculated by starter culture. The bacterial culture used determines the type of organic acids, metabolites, gases, and antibiotics released, which contribute to the fermented food's flavour, colour, aroma, and medicinal potency. Lactobacilli probiotic strains are most commonly found in fermented milk products. (Bernardeau et al., 2006; Farahmand et al., 2021).

To put it simply, microbes act on dietary items prior to consumption through fermentation, which increases the specific nutrient or phytonutrient content of foods, and these fermented dietary items influence our own microbiota. the ultimate value of which is linked to health. Various studies have demonstrated the obvious role of fermented foods in modulating the gut environment, which is linked to overall well-being.

Fermented foods may also lessen the severity of diarrhea, bloating, gas, and constipation (Hao et al., 2011). Fermented foods also aid in the absorption of essential minerals such as vitamin C, iron, and zinc, all of which have been linked to a stronger immune system (Hemilä and Chalker, 2013). Fermentation breaks down the natural sugar in milk, lactose, to glucose and galactose, making milk-based fermented beverage easy to digest for lactose intolerant patients who can't digest milk (Hertzler and Clancy, 2003).

There is a strong link between the consumption of milk-based fermented beverages

and a variety of diseases, including neurodegenerative disorders (Selhub et al., 2014). The major bacteria found in fermented milk, *Lactobacillus helveticus* and *Bifidobacterium longum*, have psychotropic properties. This has paved the way for the treatment of mental health disorders with milk-based fermented beverages.

Lactic acid bacteria proteolytic systems found in fermented milk, such as *Lactococcus lactis* and *Lactobacillus helveticus*, benefit the brain not only by maintaining gut integrity or by releasing various neurochemicals and SCFA, but they are also capable of releasing bioactive peptides from milk protein, particularly casein, which promotes various physiological functions primarily by targeting GABA receptors. This discovery was made after scientists investigated why babies become calm when breastfed (Clare and Swaisgood, 2000). These biogenic metabolites make the products directly beneficial without the need of live bacteria (Korhonen and Pihlanto, 2003). Milk contains approximately 3.5% protein of which 80% are casein and 20% whey proteins. CN consists of αs1-, αs2-, β- and k-CN families in the approximate ratio 38:11:38:13 (Pessione, 2012).

2.13 The role of biologically active peptides derived from fermented milk on brain's health

Biologically active peptides derived from milk are initially found in inactive form within the sequence of the precursor molecules, but they can be released in three ways:

i) Enzymatic hydrolysis with digestive enzymes such as pepsin, trypsin, and chymotrypsin.
ii) Fermentation of milk with proteolytic starter cultures.
iii) Proteolysis by enzymes derived from proteolytic microorganisms (Korhonen and Pihlanto, 2003).

After these bioactive peptides are released, they have an impact on a variety of health responses, including cardiovascular, digestive, endocrine, immune, and neurological activity, among others (Korhonen and Pihlanto, 2003). Animal and human studies have revealed that some milk-derived biogenic metabolites represent a promising frontier for treating stress-related behaviours such as anxiety, mood disorder, and depression, as well as for inducing relaxation and

lowering cortisol levels (Takano, 2002). When compared to controls, biopeptides were found to be significantly more effective than St. John's wort and kava kava in reducing anxiety in one double blind study (Pfluger et al., 2012).

The first major opioid peptides discovered in cow's milk after incubation with lactic acid bacteria were ß-casomorphin, which are fragments of ß-casein (Bhat and Bhat, 2015)). They are the most well-studied myorelaxant peptides (which act on mu receptors); another well-known one is alpha s1 casein-derived peptide (which acts on the delta receptor (Loukas et al., 1983). Once in the bloodstream, BM activates receptors in the brain that mediate the biological effect by activating serotonin, dopamine, and GABA (Mizushige et al., 2013). Supplementing Lactobacillus plantarum strain PS12 has reported increase in dopamine and serotonin and decrease depression (Liu et al., 2018). Associate professor of medicine at UCLA in 2013, conducted a study with 36 women. Over the course of four weeks, they were given either a milk product supplemented with probiotics, milk without probiotics, or no intervention. The researchers used fMRI to scan and monitor brain activity, as well as a face-matching attention task. During the experimental task, the probiotic supplement group had less activity in several areas of the brain. They specifically note changes in the periaqueductal grey region of the midbrain, a region of the midbrain involved in pain regulation (Tillisch et al.,2011).

2.14 Mechanism of action of psychobiotics

As seen in Figure 2.29 blue arrows represent psychobiotics processes and effects, while red arrows represent leaky gut and inflammation processes. Probiotics directly introduce beneficial bacteria into the gut, such as Lactobacilli and Bifidobacteria. Prebiotics promote the growth of these bacteria. Probiotics and prebiotics both increase the production of short-chain fatty acids (SCFAs), which interact with enteroendocrine cells in the gut and catalyze the release of gut hormones such as cholecystokinin (CCK), peptide tyrosine tyrosine (PYY), and glucagon- like peptide-1 (GLP-1). In contrast to probiotics, prebiotics can have a stronger impact in this regard. SCFAs and gut hormones are absorbed into the bloodstream and can travel to the brain. Enteroendocrine cells aren't the only ones that secrete gut hormones. **Neurotransmitters**: Psychobiotics increase the synthesis of neurotransmitters such as dopamine (DA), serotonin (5-HT),

noradrenaline (NA), and -aminobutyric acid (GABA) in the stomach, which are thought to modulate neurotransmission in the enteric nervous system's proximal synapses. **Vagal connections**: the vagus nerve synapses on enteric neurons and enables gut– brain communication. **Stress, barrier function, and cytokines**: Stress-induced glucocorticoid exposure exacerbates barrier dysfunction. This allows bacteria with pro-inflammatory components to migrate, resulting in increased inflammation and a rise in pro-inflammatory cytokines through the immunogenic response. These cytokines compromise the blood–brain barrier, allowing pathogenic or inflammatory elements to enter the brain. Pro-inflammatory cytokines (red circles) also compromise the gut barrier's integrity. Psychobiotics activity improves gut barrier function and lowers glucocorticoid and pro-inflammatory cytokinelevels in the blood (Sarkar et al., 2016).

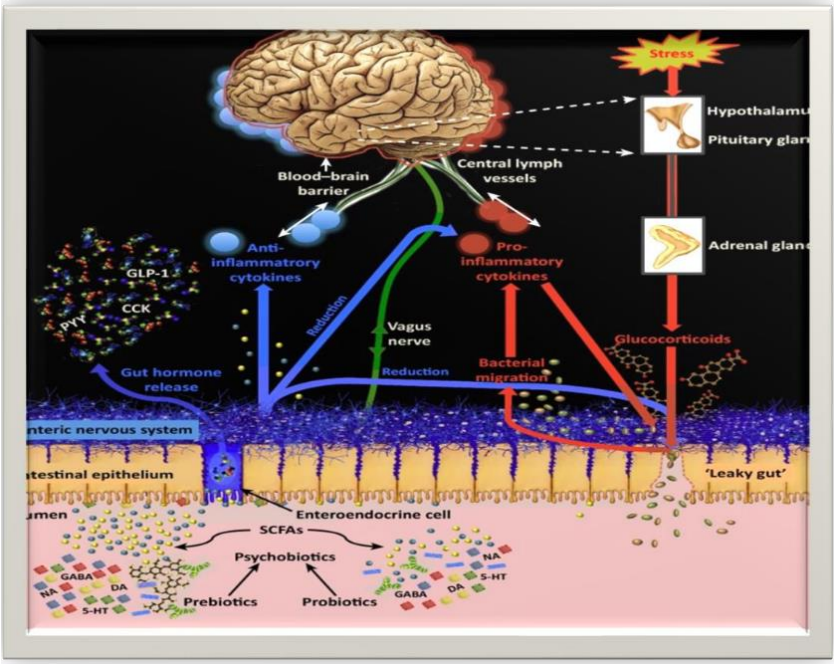

Figure 2.29: Psychobiotics strategy in gut brain axis