Cognitive impairment is evident in 'non-user' first-degree relatives of persons with cannabis dependence syndrome: An endophenotype study.

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Abstract

Introduction- Cannabis use disorders are global emerging problem nowadays, with high prevalence and morbidity. Though cognitive impairments are one of the most replicated findings in individuals with cannabis dependence, but there are very few studies assessed cognitive functioning as a risk factor for cannabis use disorder. In this study, we assessed cognitive functioning as an endophenotype in cannabis use disorders. Methodology- In this study comparison of cognitive functioning was done among three groups- patients with cannabis dependence syndrome, their first degree relative (FDR) and normal healthy controls (HC). Each group included 30 participants. Individuals of all three groups were assessed in domains of complex attention, executive functions, language, learning and memory and perceptual-motor. Results- Performance of patients with cannabis dependence was impaired in attention, verbal memory, executive functions compared to both other groups. Attention, semantic verbal fluency and memory were found to be an endophenotype as both patient and FDR group performed poorly than HC group. Verbal memory was impaired in patients' group compared to group of first-degree relatives, whose performance in-turn impaired than normal healthy controls. Performances of verbal and visual memory were correlated positively with age of onset and negatively with frequency of cannabis intake. Age of first-degree relatives was inversely correlated with verbal memory. Conclusion- Performance of individuals with cannabis dependence was impaired than normal healthy controls in all domains of cognitive functioning. As per definition, verbal memory could be considered as an endophenotype marker in cannabis use disorders.

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Results- Performance of patients with cannabis dependence was impaired in attention, verbal memory, executive functions compared to both other groups. Attention, semantic verbal fluency and memory were found to be an endophenotype as both patient and FDR group performed poorly than HC group. Verbal memory was impaired in patients' group compared to group of first-degree relatives, whose performance in-turn impaired than normal healthy controls. Performances of verbal and visual memory were correlated positively with age of onset and negatively with frequency of cannabis intake. Age of first-degree relatives was inversely correlated with verbal memory.

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1. Introduction- Cannabis is a widely used psychoactive substance all over the world. According to a global epidemiological study by Degenhardt et al. (2013), point prevalence of cannabis use disorders was 0.2% and it contributed to 0.08% of total DALY loss. A recent epidemiological survey revealed 2.8% of Indian population was currently using cannabis whereas 0.25% met diagnosis of cannabis dependence syndrome (Ambekar et al., 2019).

Cannabis use modulates certain neuro-physiological changes; through activation of cannabinoid (mainly type 1, CB1) receptors. These CB1 receptors were widely distributed in central nervous system and implicated in second messenger systems, protein signaling pathways, reward pathways, regulation of some neurotransmitters like GABA and dopamine (Kinsey and Lichtman, 2019). Widespread action might be responsible for dependence or psychological effect. Among many risk factors, genetics play an important role in precipitating dependence and other psychological effect of cannabis. There are certain genetic polymorphisms which (The catechol-O-methyltransferase gene: COMT (Henquet et al., 2006; Tunbridge et al., 2015; Freyberg et al., 2010), The AKT serine/threonine kinase 1 gene: AKT1 (Niizuma et al., 2009; Ozaita et al., 2007; Sánchez et al., 2003; Bhattacharyya et al., 2014; Hess et al., 2009), The dopamine ß-hydroxylase gene: DBH (Zabetian et al., 2001; Ramaekers et al., 2016; Sharpley et al., 2014), The serotonin transporter gene: 5-HTT/SLC6A4) (Canli and Lesch, 2007; Verdejo-García et al., 2013; Dalley et al., 2004) predispose individual with cannabis use to dependence or psychosis or cognitive impairment. So, there may be chance of familial predisposition of cannabis use.

Use of psychoactive substances could impair brain circuits responsible for executive control (Dalley et al., 2004; Goldstein and Volkow, 2002), specifically response inhibition (Fillmore and Rush, 2002; Monterosso et al., 2005), mental planning (Ersche et al., 2006; Ornstein et al., 2000), working memory (Tomasi et al., 2007; Ersche et al., 2011a; Fernández-Serrano et al., 2010), and attention control (Gooding et al., 2008; Ersche et al., 2011b; London et al., 2005). There are a number of studies regarding effect of cannabis on cognitive functioning (Cami and Farré, 2003; Fletcher et al., 1996; Pope and Yurgelun-Todd, 1996; Whitlow et al., 2004; Solowij et al., 2002; Grant et al., 2003; Croft et al., 2001; Bolla et al., 2002; Bartholomew et al., 2010). Cannabis use was found to be associated with impairment in cognitive domains such as- sustained attention, response monitoring, decision making and memory. Most of the studies emphasized heavy and long term use of cannabis for such impairments (Cami and Farré, 2003; Fletcher et al., 1996; Pope and Yurgelun-Todd, 1996; Whitlow et al., 2004; Croft et al., 2001). Heavy use of cannabis and related neuro-anatomical and neuro-physiological changes (Arnone et al., 2008; Zalesky et al., 2012) were reported as predictors of cognitive impairments in some studies (Bolla et al., 2002; Arnone et al., 2008; Zalesky et al., 2012). Specially, learning and memory deficits were impaired in heavy and long term cannabis users, which was in tandem with hippocampal attrition (Grant et al., 2003; Yucel et al., 2008). Pope et al (1996) reported residual cognitive effect in patients with cannabis dependence even in abstinence, though it was negligible after 28 days of abstinence. Fontes et al (2011) reported cognitive impairments were inversely correlated with age of onset of cannabis use. In spite of advance neurobiological findings, biological underpinning of cannabis use disorders is still obscure. Emerging data suggested that biological relatives of patients with substance use

have higher risk of developing drug dependence in future (Merikangas et al., 1998; Ersche et al., 2012).

So, the possibility of pre-morbid risk of drug dependence cannot be ruled out. Identification of biological vulnerability markers provides a scientific basis for development of effective preventive and therapeutic strategies for individuals at risk.

Studies are lacking in the field of preexisting vulnerability in addiction especially in cannabis use disorders.

The concept of endophenotype offers a useful strategy for evaluating the underlying factors that makes an individual vulnerable to any psychiatric disorder as well as substance use. Endophenotype have been defined as quantitative traits that are intermediate between the predisposing genes (genotype) and the clinical symptoms (phenotype) of a complex disorder. According to the criteria outlined by Gottesman and Gould, endophenotypes are quantifiable traits which 1) associated with the disorder; 2) genetically determined; 3) largely state independent (i.e., they should manifest in periods of health and during acute illness); 4) segregate with the disorder within families; and 5) overrepresented in unaffected family members relative to the general population (Gottesman and Gould, 2003; Gould and Gottesman, 2006). Based on proximity of deficit, endophenotypes are divided further into two levels- 'level 1'- degree of deficit in FDR group is almost similar to patient probands; and 'level 2'- degree of deficit in patient group is impaired significantly than FDR group (Tikka et al., 2015).

In our study, cognitive functions were assessed as a putative endophenotype for cannabis dependence. Relation of cognitive functioning and cannabis exposure could be bidirectional; such as, impaired cognitive functioning could be result of chronic or early cannabis exposure, or impaired cognitive functioning makes an individual vulnerable for cannabis use.

So, in this study the mentioned domains of cognition were assessed and comparison was done among three groups- patients with cannabis dependence, their un-affected first-degree relatives and normal healthy controls.

2. Methods- The study was done in a tertiary care hospital in central India. Patients were recruited from outpatient and in-patient in department of psychiatry attending in the hospital. The study was done among three groups, patients with cannabis dependence syndrome, their first-degree relatives (FDR) and normal healthy controls (HC). 30 participants were included in each group (95%CI and 65%Power). For participants of all three groups' age range remained restricted to 18 to 45 years and 8 years of formal education. Patients were included as per ICD 10 diagnostic criteria for cannabis dependence. In our study patients with cannabis dependence were included who had positive urine screening for cannabis as well as on the basis of self-reporting. All participants in FDR and HC groups were screened by GHQ 5 to rule out any mental disorder. Moreover, any participant of all three groups was excluded to take part in the study if dependence criteria of any substance were fulfilled except tobacco and caffeine. Participants from HC groups were excluded if they had any family history of Alzheimer's disease, mental retardation & organic brain disease, substance use disorder (except for tobacco & caffeine). Informed consent was taken from each participant (Flowchart shown in Fig 1).

Primary objective of this study was to assess and compare cognitive functioning among patients with cannabis dependence syndrome, first degree relatives & normal healthy controls. Secondary objective was to look for association and correlation between the degree of cognitive functioning in three groups (patients with cannabis dependence syndrome, first degree relatives & normal healthy controls) and their socio-demographic & clinical variables.

Attention and concentration, language, memory and executive functions were tested across three groups (Table no 1).

3. Data analysis-

42 patients with cannabis dependence were approached. The patients with unreliable history, not accompanied by FDR, with altered consciousness were excluded. After 20 subjects are included, along with their

FDR; an interim analysis was done. 12 patients were excluded after interim analysis as their mean age was not in sync with mean age of FDR group. As per plan, all participants were group matched with respect to age, education and gender. In this study SPSS version 15.0software was used. Shapiro-Wilk test was done to check whether continuous variables were in normal distribution. Most of our continuous variables including age, income and scores of cognitive functioning followed a skewed distribution, and so non-parametric tests were used. Kruskal-Wallis tests were used to delineate significant difference across three groups. For post-hoc test serial Mann-Whitney tests were used.

4. Results-

4.1 Socio-demographic data- Three groups were matched in terms of gender, marital status, religion, residence but there is significant difference in education (p=0.011) and occupation (0.000) (Table no 3). Mean age of patients' group with cannabis dependence was 30.57±12.90, and there was no significant difference with groups of FDR and HC (Table no 2).

4.2 Cognitive functions- Performance of attention task (digit forward and digit backward) was significantly varied across three groups ($p<0.05$ for both the test). Post-hoc test revealed performance of digit forward in patients with cannabis use was significantly impaired from normal healthy control group, though there was no significant difference between group of FDR and HC. In digit backward test, performance of patients with cannabis use was significantly lower from normal healthy control. Performance of FDR group was reduced from HC group but no significant difference between performance of patients and FDR group (Table no 4).

In verbal memory test especially phonemic test, performance of patients with cannabis use was impaired than FDR group, whose performance in turn impaired from HC group. Though in categorical verbal memory, patients with cannabis use performed worse than FDR and HC group, but no significant difference was found between later two groups (Table no 4).

In our study visual memory was not differed significantly across three groups (Table no 4).

In tests for executive functioning, performance of patients with cannabis dependence was impaired compared to normal control as well as their FDR. Significant difference ($p<.05$) was found in mistakes of Stroop test and time for Trail-A test. But there was no significant difference between FDR and HC group (Table no 4).

4.3 Correlation of socio-demographic and clinical variables with cognitive functioning-

In our study visual copy score was correlated with cannabis frequency and verbal memory deficit was correlated with frequency of cannabis. Verbal memory deficit was negatively correlated with age of onset of cannabis use (Table no 5).

4.4 Endophenotype - Patients with cannabis users have impairment in attention, verbal fluency, verbal and visual memory, executive function compared to HC group suggesting that these domains are 'disease markers**.** Subsequently, we found that FDR group performed poorly than HC group in attention (digit backward), semantic verbal fluency and verbal memory; therefore, qualifying for the definition of endophenotype (Figure no 2). Among these parameters, attention and verbal fluency was found close to illness, where performance of FDR was comparable to patients with cannabis use, therefore, qualifying for being a 'level-1' endophenotype while verbal memorywere considered 'level-2 endophenotype.

5. Discussion- We intended to identify candidate cognitive endophenotype for cannabis dependence.

Significant impairment was found in all domains of cognition (attention, verbal fluency, verbal and visual memory, executive function) in patients with cannabis dependence compared to normal healthy controls. Our findings were in sync with previous studies (Cami and Farré, 2003; Fletcher et al., 1996; Pope and Yurgelun-Todd, 1996; Whitlow et al., 2004; Solowij et al., 2002; Grant et al., 2003; Croft et al., 2001). In our study, group of patients with cannabis dependence included all 30 male participants. It would be better if we include female subjects too, but availability was the main limiting factor. To overcome gender as a confounding factor, we have tried not to include any female as a participant in other 2 groups. Across some studies it was found that males are better in visuo-spatial ability whereas females outperform in memory and

language (Kieseppa et al., 2005). A study by Bloomfield et al. (2014) assessing cerebral glucose metabolism in cannabis users found that there were significant group differences at baseline frontal metabolism between male and female. Female group showed significant attenuation of regional brain metabolic responses to methylphenidate (dopamine enhancing agent) (Bloomfield et al., 2014). The gender differences suggested that females might be more sensitive to the adverse effects of cannabis in brain. Though in another study, sex differences in cognitive performance were not significant (Bolla et al., 2002).

Attention was assessed across a number of studies and found impaired in patients with cannabis use. Assessment tools were varied across studies, for e.g. digit symbol substitution task (DSST), immediate and delayed digit recall task (DRT) (Ramesh et al., 2013), Useful Field of View (UFOV) task, trail making task (Anderson et al., 2010). A study by Anderson et al (2010) found impaired attention in cannabis users even in frequency of 1-10times/month. In verbal fluency tests, our results replicated findings of study by Pope et al (2003); though in later study there was no significant difference between late onset users (<17 years) and control groups (Anderson et al., 2010). A study by Bolla et al (2002) revealed impaired verbal memory even after 28 days of abstinence from cannabis, compared to non-users. Themes et al (2014) reported more impairment of verbal memory in recent users compared to past users, performance of whom in turn reduced than non-users. In our study we found verbal memory deficit was negatively correlated with age of onset of cannabis use, which is in sync with results of previous studies (Solowij et al., 2011). Bolla et al (2002) found impairment using Rey complex figure—copy test, which is similar to our study and found dose related impairment in patients with cannabis use. There were studies (Thames et al., 2014; Fried et al., 2005) which replicated this finding. Thames et al (2014) used similar tools of our study (Trail making test and Stroop test) to assess executive function and found impairment in cannabis users, especially in recent users. Though assessment tools for executive functions varied like Wisconsin Card Sorting Test and Continuous Performance test (Pope et al., 2003) but the finding were consistent across studies. Besides, cognitive impairment was found more impaired in lower cognitive reserve subjects (Bolla et al., 2002), though this is not a much replicated finding across studies.

Endophenotype refers to certain phenotype (such as here cognitive functioning), which corresponds to certain genes. Here, the functional consequences of risk alleles have been assessed (cognitive functioning) rather than risk gene itself. So, susceptibility gene as well as its associated neurocognitive variables may act as predisposing factor for cannabis use disorders. Patients with cannabis use performed poorly than HC group, which makes it a disease marker; suggesting the possibility of cannabis related impairment in verbal memory. Performance of FDR group in attention, semantic verbal fluency and memory was found inferior to HC group, which fulfills definition of endophenotype. Attention and verbal fluency fulfilled the definition of level 1 endophenotype which is symptom related and may be co-segregated in families. Verbal memory of FDR group lied between patients and HC group, which defined it as a level 2 endophenotype. So, verbal memory impairment was found as symptom which has a segregated genetic pool and independent of disease (state) condition (Tikka et al., 2015).

Some studies previously found impairment of cognitive domains in unaffected biological siblings of substance use disorders. Smith DG et al (2013) assessing executive functioning in stimulant use disorders found significant impairment in siblings of patient compared to normal healthy control. This findings suggested premorbid cognitive impairment might be there to precipitate drug dependence; along with-it impairment of patient group more than siblings group suggested drug induced impairment in cognitive functions. Similar findings were reported by Ersche et al (2012) in patients with stimulant use disorder. Besides, it replicated in patients with alcohol use disorders (Tunbridge et al., 2015; Bo et al., 2019; Tarter et al., 2003). A longitudinal study (Dawes et al., 1997) found that children of patients with alcohol use disorder had poor inhibitory control, which might predict substance use in them. An original study (Euser et al., 2013) explored Error Related Negativity in offspring of individuals with cannabis use disorders and found impairment in them compared to offspring of healthy control group. This could be explanatory in view of deficits in the ability to self-monitor, ongoing behavior for errors or unsuitable actions, arguments; probably because of reduced error salience. So, our study findings were consistent with this study and explored possibility of cognitive endophenotype in cannabis use disorders.

Our study has certain limitations. Power is significantly less because of small sample size. We have included patients with cannabis dependence and assessed their cognitive functions, while other co-morbidities (both physical and psychiatric) were not ruled out in this study. It is a major limitation of this study as in psychiatric disorders, cognitive functioning may be hampered irrespective of substance use. It would be better if various forms (like edible, smoked, intravenous) of cannabis were included, as this could confound the findings. Analysis according to age of onset of cannabis use is lacking in our results because of small sample size. As it is a cross-sectional study so longitudinal relationship between cannabis use and cognitive functioning could not be explored.

As this was a pilot study, which pointed towards possible endophenotype in cannabis use disorders, it can be performed in large sample size. In case of any established cognitive endophenotype, primary prevention of cannabis use disorders may be done for defined population. Unaffected biological relatives should have cognitive screening and further rehabilitation according to their status. Further, psycho-education should be given to unaffected first-degree relatives of patients with cannabis dependence about harmful effects of cannabis and risk of precipitating cannabis use disorders; monitoring for early signs should be explained to them.

References-

- Ambekar, A., Agrawal, A., Rao, R., Mishra, A.K., Khandelwal, S.K., Chadda, R.K., 2019. Magnitude of substance use in India. National Drug Dependence Treatment Centre, New Delhi, Government of India. Ministry of Social Justice and Empowerment.
- Anderson, B.M., Rizzo, M., Block, R.I., Pearlson, G.D., O'Leary, D.S., 2010. Sex, drugs, and cognition: effects of marijuana. Journal of Psychoactive Drugs, 42, pp.413-424.
- Arnone, D., Barrick, T.R., Chengappa, S., Mackay, C.E., Clark, C.A., Abou-Saleh, M.T., 2008. Corpus callosum damage in heavy marijuana use: preliminary evidence from diffusion tensor tractography and tract-based spatial statistics. *NeuroImage* , 41, pp.1067–1074.
- Bartholomew, J., Holroyd, S., Heffernan, T.M., 2010. Does cannabis use affect prospective memory in young adults? *Journal of Psychopharmacology* , 24(2), pp.241-246.
- Bhattacharyya, S., Iyegbe, C., Atakan, Z., Martin-Santos, R., Crippa, J.A., Xu, X., et al., 2014. : Protein kinase B (AKT1) genotype mediates sensitivity to cannabis-induced impairments in psychomotor control. Psychological Medicine, 44(15), pp.3315-28.
- Bloomfield, M.A., Morgan, C.J., Egerton, A., Kapur, S., Curran, H.V., Howes, O.D., 2014. Dopaminergic function in cannabis users and its relationship to cannabis- induced psychotic symptoms. *Biological Psychiatry,* 75, pp.470–478.
- Bo, Q., Dong, F., Li, X., Li, F., Li, P., Yu, H., et al., 2019. Comparison of cognitive performance in bipolar disorder, major depressive disorder, unaffected first-degree relatives, and healthy controls. *Psychiatry Clinical Neurosciences* , 73(2), pp.70-76.
- Bolla, K.I., Brown, K., Eldreth, D., Tate, K., Cadet, J.L., 2002. Dose-related neurocognitive effects of marijuana use.*Neurology* , 59, pp.1337–1343.
- Cami, J.,Farre, M., 2003. Mechanisms of drug addiction - Reply.*New England Journal of Medicine.* 349, pp.2365-2365.
- Canli, T., Lesch, K.P., 2007. : Long story short: the serotonin transporter in emotion regulation and social cognition. Nature Neuroscience, 10(9), pp.1103-9.
- Croft, R.J., Mackay, A.J., Mills, A.T.D., Gruzelier, J.G.H., 2001. The relative contributions of ecstasy and cannabis to cognitive impairment. *Psychopharmacology,* 153, pp.373–379.
- Dalley, J.W., Cardinal, R.N., Robbins, T.W., 2004.: Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neuroscience and Biobehavioral Reviews* , 28, pp.771–784.
- Dawes, M.A., Tarter, R.E., Kirisci, L., 1997. Behavioral self-regulation: correlates and 2-year follow-ups for boys at risk for substance abuse. *Drug and Alcohol Dependence,* 45, pp.165–176.
- Degenhardt, L., Ferrari, A.J., Calabria, B., Hall. W.D., Norman, R.E., McGrath, J., et al., 2013. The Global Epidemiology and Contribution of Cannabis Use and Dependence to the Global Burden of

Disease: Results from the GBD 2010 Study. *PLoS ONE* , 8(10), e76635.

- Ersche, K.D., Barnes, A., Jones, P.S., Morein-Zamir, S., Robbins, T.W., Bullmore, E.T., 2011b. : Abnormal structure of frontostriatal brain systems is associated with aspects of impulsivity and compulsivity in cocaine dependence. *Brain,* 134, pp.2013–2024.
- Ersche, K.D., Clark, L., London, M., Robbins, T.W., Sahakian, B.J., 2006. Profile of executive and memory function associated with amphetamine and opiate dependence. *Neuropsychopharmacology,* 31, pp.1036–1047.
- Ersche, K.D., Roiser, J.P., Lucas, M., Domenici, E., Robbins, T.W., Bullmore, E.T., 2011a. : Peripheral biomarkers of cognitive response to dopamine receptor agonist treatment. *Psychopharmacology,*214, pp.779–789.
- Ersche, K.D., Turton, A.J., Chamberlain, S.R., Muller, U., Bullmore, E.T., Robbins, T.W., 2012. Cognitive dysfunction and anxious-impulsive personality traits are endophenotypes for drug dependence.*American journal of Psychiatry,* 169(9), pp.926-936.
- Euser, A.S., Evans, B.E., Greaves-Lord, K., Huizink, A.C., Franken, I.H., 2013. Diminished error-related brain activity as a promising endophenotype for substance-use disorders: evidence from high-risk offspring. *Addiction Biology,* 18(6), pp.970-84.
- Fernandez-Serrano, M.J., Perez-Garcia, M., Schmidt, R.J., Verdejo-Garcia, A., 2010. : Neuropsychological consequences of alcohol and drug abuse on different components of executive functions.*Journal of Psychopharmacology,* 24, pp.1317–1332.
- Fillmore, M.T., Rush, C.R., 2002.: Impaired inhibitory control of behavior in chronic cocaine users. *Drug and Alcohol Dependence,*66, pp.265–273.
- Fletcher, J.M., Page, J.B., Francis, D.J., Copeland, K., Naus, M.J., Davis, C.M., et al., 1996. Cognitive correlates of long-term cannabis use in Costa Rican men. *Archives of general psychiatry* , 53(11), pp.1051-1057.
- Fontes, M.A., Bolla, K.I., Cunha, P.J., Almeida, P.P., Jungerman, F., Laranjeira, R.R., et al., 2011. Cannabis use before age 15 and subsequent executive functioning. *British Journal of Psychiatry* , 198, pp.442–447.
- Freyberg, Z., Ferrando, S.J., Javitch, J.A., 2010. : Roles of the Akt/GSK-3 and Wnt signaling pathways in schizophrenia and antipsychotic drug action. American Journal of Psychiatry, 167(4), pp.388-96.
- Fried, P.A., Watkinson, B., Gray, R., 2005. Neurocognitive consequences of marijuana – a comparison with pre-drug performance. Neurotoxicolology and Teratology, 27, pp.231-239.
- Goldstein, R.Z., Volkow, N.D., 2002. Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. *American Journal of Psychiatry* , 159, pp.1642–1652
- Gooding, D.C., Burroughs, S., Boutros, N.N., 2008. Attentional deficits in cocaine-dependent patients: converging behavioral and electrophysiological evidence. *Psychiatry Research,* 160, pp.145–154.
- Gottesman, I.I., Gould, T.D., 2003.The endophenotype concept in psychiatry. Etymology and strategic intentions. *American Journal of Psychiatry* , 160, pp.636–645.
- Gould, T.D., Gottesman, I.I., 2006.Psychiatric endophenotypes and the development of valid animal models. *Genes, Brain and Behavior* , 5, pp.113–119.
- Grant, I., Gonzalez, R., Carey, C.L., Natarajan, L., Wolfson, T., 2003. Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. *Journal of the International Neuropsychological Society,* 9, pp.679–689.
- Henquet, C., Rosa, A., Krabbendam, L., Papiol, S., Fananas, L., Drukker, M., et al., 2006. : An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9- tetrahydrocannabinol-induced effects on psychosis and cognition. Neuropsychopharmacology, 31(12), pp.2748-57.
- Hess, C., Reif, A., Strobel, A., Boreatti-Hummer, A., Heine, M., Lesch, K.P., et al., 2009. : A functional dopamine-beta-hydroxylase gene promoter polymorphism is associated with impulsive personality styles, but not with affective disorders. Journal of Neural Transmission, 16(2), pp.121-30.
- Kieseppa, T., Tuulio-Henriksson, A., Haukka, J., Van Erp, T., Glahn, D., Cannon, T.D., et al., 2005. Memory and verbal learning functions in twins with bipolar-I disorder, and the role of information-

processing speed. *Psychological Medicine* , 35, pp.205–215.

- Kinsey, S., Lichtman, S., 2019. The Endogenous Cannabinoid System: A Cadre of Potential Therapeutic Targets, in: Montoya I, Weiss S (eds). *Cannabis use disorders* , Springer International Publishing, Switzerland, pp.21-29.
- London, E.D., Berman, S.M., Voytek, B., Simon, S.L., Mandelkern, M.A., Monterosso, J., et al., 2005. : Cerebral metabolic dysfunction and impaired vigilance in recently abstinent methamphetamine abusers.*Biological Psychiatry* , 58, pp.770–778.
- Merikangas, K.R., Stolar, M., Stevens, D.E., Goulet, J., Preisig, M.A., Fenton, B., et al., 1998. : Familial transmission of substance use disorders. *Archives of General Psychiatry,* 55, pp.973–979.
- Monterosso, J.R., Aron, A.R., Cordova, X., Xu, J., London, E.D., 2005.: Deficits in response inhibition associated with chronic methamphetamine abuse. *Drug and Alcohol Dependence* , 79, pp.273–277.
- Niizuma, K., Endo, H., Chan, P.H., 2009. : Oxidative stress and mitochondrial dysfunction as determinants of ischemic neuronal death and survival. Journal of Neurochemistry, , 109, pp.133-8.
- Ornstein, T.J., Iddon, J.L., Baldacchino, A.M., Sahakian, B.J., London, M., Everitt, B.J., et al., 2000. Profiles of cognitive dysfunction in chronic amphetamine and heroin abusers.*Neuropsychopharmacology* , 23, pp.113–126.
- Ozaita, A., Puighermanal, E., Maldonado, R., 2007. : Regulation of PI3K/Akt/GSK-3 pathway by cannabinoids in the brain. Journal of Neurochemistry, 102(4), pp.1105-14.
- Pope, H.G., Gruber, A.J., Hudson, J.I., Cohane, G., Huestis, M.A., Yurgelun-Todd, D., 2003. Early-onset cannabis use and cognitive deficits: what is the nature of the association? *Drug and Alcohol Dependence* , 69(3), pp.303-310.
- Pope, H.G., Yurgelun-Todd, D., 1996. The residual cognitive effects of heavy marijuana use. *The Journal of the American Medical Association,* 275, pp. 521–527.
- Ramaekers, J.G., van, Wel, J.H., Spronk, D., Franke, B., Kenis, G., Toennes, S.W., et al., 2016. : Cannabis and cocaine decrease cognitive impulse control and functional corticostriatal connectivity in drug users with low activity DBH genotypes. Brain Imaging and Behavior, 10(4), pp.1254-63.
- Ramesh, D., Haney, M., Cooper, Z.D., 2013. Marijuana's dose-dependent effects in daily marijuana smokers. Experimental and Clinical Psychopharmacology, 21, pp.287-293.
- Sanchez, M.G., Ruiz-Llorente, L., Sanchez, A.M., Diaz-Laviada, I., 2003. : Activation of phosphoinositide 3-kinase/PKB pathway by CB(1) and CB(2) cannabinoid receptors expressed in prostate PC-3 cells. Involvement in Raf-1 stimulation and NGF induction. Cellular Signalling, 15(9), pp.851-9.
- Sharpley, C.F., Palanisamy, S.K.A., Glyde, N.S., Dillingham, P.W., Agnew, L.L., 2014. : An update on the interaction between the serotonin transporter promoter variant (5-HTTLPR), stress and depression, plus an exploration of non-confirming findings. Behavioural Brain Research, 273, pp.89-105.
- Smith, D.G., Jones, P.S., Bullmore, E.T., Robbins, T.W., Ersche, K.D., 2013. Cognitive control dysfunction and abnormal frontal cortex activation in stimulant drug users and their biological siblings. *Translational Psychiatry,* 3(5), pp.257.
- Solowij, N., Jones, K.A., Rozman, M.E., Davis, S.M., Ciarrochi, J., Heaven, P.C., et al., 2011. Verbal learning and memory in adolescent cannabis users, alcohol users and non-users.*Psychopharmacology,* 216, pp.131-144.
- Solowij, N., Stephens, R.S., Roffman, R.A., 2002. Cognitive functioning of long-term heavy cannabis users seeking treatment.*Journal of the American Medical Association* , 287, pp. 1123–1131.
- Tarter, R.E., Kirisci, L., Mezzich, A., Cornelius, J.R., Pajer, K., Vanyukov, M., et al., 2003. Neurobehavioral disinhibition in childhood predicts early age at onset of substance use disorder. *American Journal of Psychiatry,* 160, pp.1078–1085.
- Thames, A.D., Arbid, N., Sayegh, P., 2014. Cannabis use and neurocognitive functioning in a non-clinical sample of users. Addictive Behaviors, 39, pp.994-999.
- Tikka, S.K., Nizamie, S.H., Goyal, N., Pradhan, N., Tikka, D.L., Katshu, M.Z.U.H., 2015. Evaluation of spontaneous dense array gamma oscillatory activity and minor physical anomalies as a composite neurodevelopmental endophenotype in schizophrenia. International Journal of Developmental Neuroscience. 40, 43e51.

- Tomasi, D., Goldstein, R.Z., Telang, F., Maloney, T., Alia-Klein, N., Caparelli, E.C., et al., 2007. : Widespread disruption in brain activation patterns to a working memory task during cocaine abstinence. *Brain Research,* 1171, pp.83–92.
- Tunbridge, E.M., Dunn, G., Murray, R.M., Evans, N., Lister, R., Stumpenhorst, K., et al., 2015. : Genetic moderation of the effects of cannabis: catechol-O-methyltransferase (COMT) affects the impact of Δ9-tetrahydrocannabinol (THC) on working memory performance but not on the occurrence of psychotic experiences. Journal of Psychopharmacology, 29(11), pp.1146-51.
- Verdejo-García, A., Fagundo, A.B., Cuenca, A., Rodriguez, J., Cuyás, E., Langohr, K., et al., 2013. : COMT val158met and 5-HTTLPR genetic polymorphisms moderate executive control in cannabis users. American College of Neuropsychopharmacology, 38(8), pp.1598-606.
- Whitlow, C.T., Liguori, A., Livengood, L.B., 2004. Long-term heavy marijuana users make costly decisions on a gambling task. *Drug and Alcohol Dependence* , 76, pp. 107–111.
- Yucel, M., Solowij, N., Respondek, C., Whittle, S., Fornito, A., Pantelis, C., et al., 2008. Regional brain abnormalities associated with long-term heavy cannabis use. *Archives of General Psychiatry* , 65, pp.694–701.
- Zabetian, C.P., Anderson, G.M., Buxbaum, S.G., Elston, R.C., Ichinose, H., Nagatsu, T., et al., 2001. : A quantitative-trait analysis of human plasma-dopamine beta-hydroxylase activity: evidence for a major functional polymorphism at the DBH locus. American Journal of Human Genetics, 68(2), pp.515-22.
- Zalesky, A., Solowij, N., Yu"cel, M., Lubman, D.I., Takagi, M., Harding, I.H., et al., 2012. Effect of long-term cannabis use on axonal fibre connectivity. *Brain,* 135, pp.2245–2255.

Figure no- 1

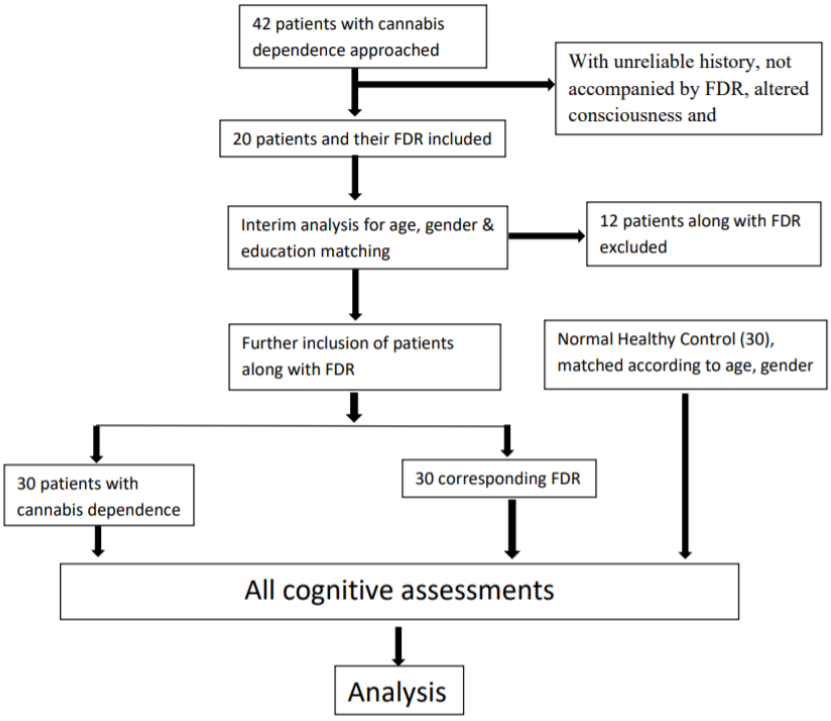

Table no-1- Cognitive assessments done in each domain

Attention & concentration	Digit forward & digit backward
Language	Verbal fluency- F-A-S test, animal naming test
Memory	Verbal memory- Rey Auditory verbal learning test Recent- orientation Remote- personal history-verified from attendant. Visual – Rey Osteirreth complex figure test.
Executive function	Stroop test, Trail making test

Table no-2- Age and income across three groups

Variables	Group- cannabis patients (n=30)	Group – FDR (n=30)	Group –HC (n=30)
	Mean± SD	Mean± SD	Mean± SD
Age (years)	30.57±12.90	30.27±13.20	27.50±5.34
Income	15050±9142.88	15050±9142.88	21170±23726.27

Table no- 3- Socio-demographic parameters across three groups

		Group-cannabis patients n=30 (%)	Group-FDR n=30 (%)	Group HC n=30 (%)	p
Gender	Male	30 (100%)	27 (90%)	30 (100%)	.104
	Female	0 (0%)	3 (10%)	0 (100%)	
Marital status	Married	15 (50%)	16 (53.3%)	21 (70%)	.24
	Single	15 (50%)	14 (46.7%)	9 (30%)	
Education	Uneducated	1 (3.3%)	1 (3.3%)	0 (0%)	.011
	Primary	7 (23.3%)	3 (10%)	0 (0%)	
	High school	16 (53.3%)	16 (53.3%)	15 (50%)	
	Intermediate	2 (6.7%)	1 (3.3%)	9 (30.0%)	
	Graduation	4 (13.3%)	8 (26.7%)	5 (16.7%)	
	PG	0 (0%)	1 (3.3%)	1 (3.3%)	
Occupation	Professional	0 (0%)	1 (3.3%)	0 (0%)	.000
	Skilled	2 (6.7%)	8 (26.7%)	17 (56.7%)	
	Semiskilled	7 (23.3%)	5 (16.7%)	7 (23.3%)	
	Unskilled	6 (20.0%)	8 (26.7%)	3 (10.0%)	
	Unemployed	15 (50.0%)	7 (23.3%)	3 (10.0%)	
	Housewife	0 (0%)	1 (3.3%)	0 (0%)	
Religion	Hindu	27 (90%)	27 (90%)	29 (96.7%)	.692
	Muslim	3 (10%)	3 (10%)	1 (3.3%)	
Residence	Rural	14 (46.7%)	14 (46.7%)	20 (66.7%)	.200
	Urban	16 (53.3%)	16 (53.3%)	10 (33.3%)	
Tobacco	Yes	27 (90%)	17 (56.7%)	16 (53.3%)	.004
	No	3 (10%)	13 (43.3%)	14 (46.7%)	

Table no- 4- Comparison of cognitive performances across three groups

Variables	Group-cannabis patients n=30 (%)	Group-FDR n=30 (%)	Group HC n=30 (%)	P	Post-hoc
Digit forward	4.87±.97	5.43±.77	5.63±.56	**.003**	**C<H,F-H,C<F**
Digit backward	3.07±.64	3.27±1.05	3.80±.66	**.001**	**C<H,F<H,C-F1**
Verbal-fluency F	3.70±1.47	4.10±1.16	6.43±1.22	**.000**	**C<H,F<H,C-F**
Verbal-fluency A	3.20±1.10	3.47±1.11	6.20±1.16	**.000**	**C<H,F<H,C-F**
Verbal-fluency S	3.40±1.45	3.78±1.14	6.37±1.00	**.000**	**C<H,F<H,C-F**
Categorical fluency	8.60±2.55	10.43±2.22	11.27±2.68	**.001**	**C<H,F-H,C<F**
Visual memory copy	26.42±10.76	29.15±7.95	32.67±5.23	**.078**	
Visual memory time	2.48±.85	2.35±.87	2.04±.96	**.094**	
RAVLT Hits	9.17±1.97	10.67±1.90	13.13±1.36	**.000**	**C<H,F<H,C<F**
RAVLT Commission	3.33±1.24	2.50±1.22	1.33±.99	**.000**	**C<H,F<H,C<F**
RAVLT Omission	5.10±1.95	4.33±1.90	1.93±1.36	**.000**	**C<H,F<H,C<F**
Stroop test score	163.50±100.42	172.07±94.73	133.00±76.21	**.288**	
Stroop mistake	4.03±2.34	2.52±1.25	2.25±.70	**.000**	**C<H,F-H,C<F**
Trail time A	58.75±32.59	38.62±10.08	42.00±28.79	**.004**	**C<H,F-H,C<F**
Trail time B	99.17±50.64	95.93±46.51	79.64±52.71	**.123**	

Table no- 5

Cannabis patient- Spearman's Correlation of socio demographic parameters with cognitive functioning

	Cognitive variables	**P**
Cannabis_onset	RAVLT, T1-5	**<.05**
Cannabis_frequency	Visual memory copy score RAVLT, T1-5	**.047 <.05**
FDR- Spearman's Correlation of socio demographic parameters with cognitive functioning	**FDR- Spearman's Correlation of socio demographic parameters with cognitive functioning**	**FDR- Spearman's Correlation of socio demographic parameters with cognitive functioning**
Age	RAVLT, T2	**.038**

Figure no- 2