# Original Article

# Use of Filter Paper to Measure Alcohol Biomarkers among Opioid-Dependent Patients on Agonist Maintenance Treatment: A Community-Based Study

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## ABSTRACT

**Background:** Harmful Alcohol use is frequent among opioid dependents patients undergoing agonist maintenance treatment. The objective assessment of harmful alcohol use can be done using laboratory measures of serum biomarkers. For community-based patients, there is often a requirement of an alternative method due to lack of onsite laboratory services. The aim of the study was to examine filter paper as a matrix to measure serum biomarkers of harmful alcohol use. **Methods:** The initial phase involved standardization of the filter-paper-based assay. Conditions were optimised for extraction and estimation of alcohol biomarkers (Aspartate Aminotransferase; AST, Alanine Aminotransferase; ALT, Gamma Glutamyl transferase; GGT and Carbohydrate Deficient Transferrin; CDT) from the filter paper. For clinical validation, serum samples were collected from community clinics. Biomarker levels obtained from both the methods were correlated using linear regression analysis. Limits of agreement between the two methods was estimated using the Intraclass Correlation Coefficient (ICC). **Results:** The extraction of enzymes (AST, ALT and GGT) from filter paper was carried out using the substrate buffer available with the reagent kit (Randox, UK). CDT was readily extracted from filter paper using deionised water. Serum biomarker levels measured from samples collected from community clinics correlated well with filter paper extracted levels (ICC 0.97-0.99). More than 90% of alcohol biomarker levels were recovered from the filter paper matrix using this method. **Conclusion:** Filter paper has the potential to be used as a matrix to objectively measure alcohol biomarkers among opioid-dependent patients from community settings lacking onsite laboratory facilities.

**Key words:** *Agonist maintenance, Alcohol biomarkers, community clinics, filter paper, opioid dependents*
**Key messages:** *Alcohol biomarkers can be measured efficiently from filter paper. The developed method may help to frequently assess the health status in patients undergoing maintenance treatment from community clinics.*

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Opioids are a major class of problem drugs, which cause significant disease burden and deaths worldwide.[1] A national survey among a treatment-seeking Indian sample reported the use of opiates as a primary drug of abuse in 26%,[2] which has risen to 53% in recent studies.[3] Long-term treatment with agonists like methadone and buprenorphine is the standard treatment available in India.[4] Patients undergoing agonist treatment frequently consume alcohol in a hazardous or dependent pattern.[5,6] This leads to adverse impacts like interactions with agonist medication, non-adherence, increased risk of fatal overdose, hepatotoxicity, and impairments in quality of life.[7] Hence, strategies to address the same become important, not only in dealing with alcohol-related problems but also in improving the treatment outcomes.[8]

Besides routine screening through self-reporting as measured by a standardized questionnaire, clinicians also need objective tools to assess the extent and pattern of alcohol use. Some of the traditional, inexpensive blood biomarkers such as aspartate aminotransferase (AST), alanine transaminase (ALT) and gamma glutamyl transferase (GGT) can be instrumental in identifying subjects with alcohol use problems. However, these biomarkers lack specificity, especially for harmful levels of use.[9] The relatively newer biomarker carbohydrate-deficient transferrin (CDT) has an important clinical value of being more sensitive to alcohol consumption *per se* rather than the effects of liver disease. The value of CDT increases at daily ethanol consumptions ranging from 40-80 g for a duration of 2-3 weeks. CDT also has sensitivity almost equal to that of GGT but is more specific.[10,11] Combining CDT and GGT tests for assessment of patients with alcohol use disorders gives a higher sensitivity (85%) than either of the assays alone.[12]

However, one of the challenges related to assessing biomarkers in clinical settings is to manage the logistics of sample collection and transportation to the laboratory. Transportation of samples to a central lab involves extra work of packaging, transportation, and labelling. Thus, it is expensive and tedious and involves problems like spillage and breakage.[13] An alternative sampling method is often looked for to carry out field-based studies. Recently, the use of filter paper for the transport of samples has gained popularity for resource-poor settings.[14,15] The use of filter paper for the collection and storage of serum has many advantages, including ease of collection and transportation. Serum samples collected on filter paper has been reported to efficiently measure various biochemical analytes, including AST and ALT.[16,17]

The study facility is a national level tertiary care treatment center for drug dependence, which is involved with various community-based projects and programs (including treatment and surveillance programs) at many places all over India. One of the challenges faced by the center is to provide support for laboratory needs in remote or densely populated areas where the facilities for performing laboratory test by a properly trained staff and in an established laboratory may not be available on the spot. The option is often that of a centralized laboratory carrying out all the investigations.

Thus, it was deemed worthwhile to explore the use of filter paper as a matrix to transport serum samples from community settings to measure alcohol biomarkers among opioid-dependent patients on agonist maintenance treatment.

## METHODS

The study was carried out in a tertiary care treatment centre for substance use disorders. Ethical issues were addressed by maintaining the confidentiality of the subjects and obtaining consent before enrolling in the study. Ethical permission was obtained from the Institute Ethics Committee. The study was carried out in two phases. The first phase involved the optimization of conditions for extraction, recovery, and analysis of biochemical markers for alcohol use from serum spotted and dried on to filter paper. The second phase was the clinical validation of the standardized filter paper method.

### Phase 1: Optimization

#### *Estimation of enzymes*

Estimation of AST, ALT and GGT was carried out from serum samples in chemistry analyzer AU 480 (Beckman Coulter), using reagents from Randox Laboratories, UK.[18] The levels obtained in direct serum samples were compared with their corresponding serum samples spotted on to filter paper.

#### *Extraction and estimation of enzymes from filter paper*

The serum-based calibrators for AST, ALT and GGT (139, 133 and 139 U/L) and controls level 1 (38, 36, 48 U/L) and level 2 (179, 123, 169 U/L) were used. A single drop of serum corresponding to 20 uL was spotted on to Whatman filter paper (903) in a non-absorbent surface. The filter disc was dried at room temperature (24-30°C) and kept at 4°C overnight. Extraction was carried out in the whole 20 uL disc. The disc was cut down to small pieces and the reagents were tried out for their extraction efficiency. Estimation of all the enzymes was carried out in chemistry analyzer AU 480 (Beckman Coulter) by small modifications in the protocol.

*Direct serum estimation of CDT*

A quantitative, sandwich enzyme immunoassay technique was used to measures CDT from serum samples.[19] The assay was performed by ELISA (Enzyme-Linked Immuno-Sorbent Assay) technique (Tecan GENios ELISA reader, Austria GmbH, Austria), using Magellan software. The procedure was followed as per the protocol provided by the manufacturer (Cusabio, USA). The concentration of CDT was determined using the professional software "Curve Expert" to make a standard curve from the web (www.cusabio.com). The levels obtained in direct serum samples were compared with their corresponding serum samples spotted on to filter paper.

*Extraction and estimation of CDT from filter paper*

Filter paper standards were prepared by spotting (20 uL) serum-based standard with CDT concentrations of 0, 50, 100, 350, 800 and 1600 ng/ml. After drying, the filter disc was kept at 4°C. The extraction conditions were standardized using various buffers under different conditions, and CDT measurement was carried out in the elute to estimate the recovery from filter paper.

### Phase 2: Clinical validation

The study was carried out in three community settings lacking laboratory facilities. Samples were collected from patients attending community clinics run by the centre for the treatment of substance use disorder in three localities of Delhi (a distance of 20 to 40 km from the laboratory). The inclusion criteria for the study were: males aged between 18 to 60 years, diagnosed with opioid dependence (ODS as per ICD-10) by a trained psychiatrist and maintained on opioid agonists for at least three months, self-reporting regular alcohol use for the past three months. Those with a file diagnosis of co-morbidity (other substance abuse or dependence except tobacco, or axis 1 psychiatric comorbidity) were excluded.

A total of 45 participants meeting the selection criteria were included. After obtaining informed consent and clinical data, a blood sample (2 ml) was drawn in serum separator vacutainers. Clinical assessment included a semi-structured questionnaire for recording socio-demographic variables (age, gender, marital status, education, and employment) and clinical variables (alcohol use, OST [opioid substitution therapy] duration, compliance, and side effects). The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST), developed for the World Health Organization, was also used for early identification of substance use disorders.[20]

The blood samples were transported to the laboratory on the same day. Serum was separated by centrifugation at 2500 rpm for 15 minutes. The analysis of serum enzymes (AST, ALT, and GGT) was carried out on the same day. Serum was spotted on Whatman filter paper 903 as per the standardized conditions, and the remaining sample was stored at -20°C for CDT estimation as per the standardized conditions. The alcohol biomarkers levels measured from direct serum were compared with filter paper levels.

### Statistical analysis

Descriptive statistics was employed to present the socio-demographic and clinical variables as number (%). The quantitative variables are summarized as mean with standard deviation (SD). The relationship between the biomarker levels obtained from serum and corresponding filter spots collected simultaneously were assessed using linear regression analysis. Intraclass correlation was calculated to estimate the limits of agreement between the two methods. The data were analyzed using IBM SPSS statistics 2015, version 20.0.

## RESULTS

### Patient characteristics: Sociodemographic and clinical details

All the participants were diagnosed with opioid dependence syndrome and maintained on OST with sublingual buprenorphine for varying length of time, with a mean (SD) of 40 (54.8) months. The mean age of the patients was 37.04 (10.7) years. Three-fourth ($n$ = 33) of the patients were married, 22% (10) were unmarried, and only 4% (2) were either divorced or widower. One-fourth (11) of the patients were just literate, one-fourth (11) were either graduates or had acquired higher education, 30% (13) had studied up to 12$^{th}$ standard, and for the rest of the patients, the information was unavailable. More than 70% (34) of the patients were employed, 18% (9) were either self-employed or doing business, and two patients were unemployed.

Self-reported alcohol use was present in 60% of the patients on a weekly basis, followed by daily and monthly use [Table 1]. All the subjects were using tobacco, and almost half of them were cannabis users. When the patients were asked about their OST compliance, one third reported very regular use (more than 24 days/month). More than 90% denied any craving for opioids.

### Extraction and estimation of biomarkers from filter paper

The extraction of serum enzymes (AST, ALT, and GGT) was effectively carried out in their respective substrate buffers. The levels obtained from quality controls compared well with their respective filter extracted

values. The extraction of CDT from filter paper serum spots was found to be optimal using distilled water. The optimal conditions for extraction of alcohol biomarkers (AST, ALT, and GGT and CDT) were standardized in filter paper spots [Table 2].

### Clinical validation for measurement of biomarkers of harmful alcohol use from filter paper

Figure 1 shows the levels measured from direct serum and their corresponding filter paper spots. The values of all the biomarkers measured from direct serum correlated well with their corresponding filter paper levels [Table 3].

## DISCUSSION

Harmful alcohol use is one of the common comorbidities associated with opioid-dependent patients on agonist maintenance treatment. This study was planned to objectively assess harmful alcohol use among opioid-dependent patients from community setting using filter paper as a matrix to measure alcohol biomarkers.

**Table 1: Clinical profile of the subjects/patients (*n*=45)**

| Clinical Profile | Number (%) (*n*=45) |
|---|---|
| Pattern of alcohol use | Daily: 10 (22) |
| | Weekly: 28 (63) |
| | Monthly: 7 (16) |
| Use of other substance | Cannabis: 20 (45) |
| | Tobacco: 45 (100) |
| Medication compliance* | Very Regular: 34 (76) |
| | Regular: 3 (6.5) |
| | Irregular: 2 (4.5) |
| | Not known: 6 (13) |
| Craving for opioids | Present: 3 (7) |
| | Absent: 42 (93) |
| Buprenorphine side effect | Present: 4 (9) |
| | Absent: 41 (91) |
| WHO ASSIST Score# | Moderate risk: 75.5 (34) |
| | High risk: 24.5 (11) |

*Very Regular (>24 day/month), Regular (15-24 day/month), Irregular (>15 day/month). #Moderate risk (11-26), High risk (27 and above), ASSIST - Alcohol, Smoking and Substance Involvement Screening Test

Routinely measured liver enzymes AST, ALT, and GGT were studied to assess the harm associated with alcohol use while CDT was included as a biomarker of alcohol use.[9,10] In community or field-based settings, the use of filter paper is associated with several advantages like ease of collection and transportation.[15] The standardization of the filter paper method was carried out as per our early reports.[16,17] Extraction of liver enzymes was efficiently carried out in their respective buffers, and the levels were measured using chemistry analyzers. Automation of the filter paper method in this study has made it adoptable and feasible.[21] CDT was found to be efficiently extracted from dried serum spots using water. Earlier, CDT estimation from dried blood spotted filter paper was reported using the electrophoresis method.[22] The present method developed for total CDT measurement is simple and fast, with one step extraction from dried serum.

The levels of biomarkers as measured in direct serum correlated well when compared with serum spotted onto filter paper. The recovery of biomarkers from filter paper was more than 90% for all the enzymes, using automated chemistry analyzer. For CDT, the recovery remained 89%, using a manual enzyme-linked immunoassay method. The two methods correlated well, with more than 0.95 ICC values. Filter paper has the potential to be used as a matrix to transport and measure alcohol biomarkers from field-based community studies. Previous studies from our group and other authors also reported filter paper as a reliable matrix for biochemical measurements.[13-17]

**Table 2: Standardized conditions for alcohol biomarkers measurements from filter paper**

| Biomarker | Serum spot volume | Extraction agent | Extraction Condition | Estimation condition (volume µl) | Instrumentation |
|---|---|---|---|---|---|
| AST, ALT and GGT | 20 µl | 200 µl substrate buffer | Vortex briefly. Spin at 4000 rpm for 1 min. Repeat twice | Sample: 10<br>R1: 125<br>R2: 25 | Chemistry analyzer |
| CDT | 10 µl × 5 spots | 300 µl water | 1-h extraction at 37°C with brief vortexing | 50 | ELISA Reader |

AST – Aspartate aminotransferase, ALT – Alanine aminotransferase, GGT – Gamma gutamyl transferase, CDT – Carbohydrate deficient transferrin, R1 – Reagent 1, R2 – Reagent 2

**Table 3: Comparison between levels of alcohol biomarkers measured from direct and filter paper method**

| Alcohol Biomarker | Direct levels Mean (SD) | FP levels Mean (SD) | Recovery % | $R^2$* | ICC# (*P*) |
|---|---|---|---|---|---|
| AST | 50.07 (81.9) (U/L) | 49.9 (83.47) (U/L) | 99 | 0.95 | 0.97 (0.001) |
| ALT | 59.57 (56.57) (U/L) | 53.2 (57.13) (U/L) | 96.5 | 0.98 | 0.99 (0.001) |
| GGT | 51.89 (61.70) (U/L) | 49.9 (60.69) (U/L) | 96.2 | 0.99 | 0.99 (0.001) |
| CDT | 531.84 (518.88) (ng/ml) | 474.95 (495.16) (ng/ml) | 89 | 0.99 | 0.99 (0.001) |

*$R^2$ Goodness of fit in linear regression between two variables. #ICC Intra class correlation between two variables. AST – Aspartate aminotransferase. AL + T – Alanine aminotransferase, GGT – Gamma gutamyl transferase, CDT – Carbohydrate deficient transferrin

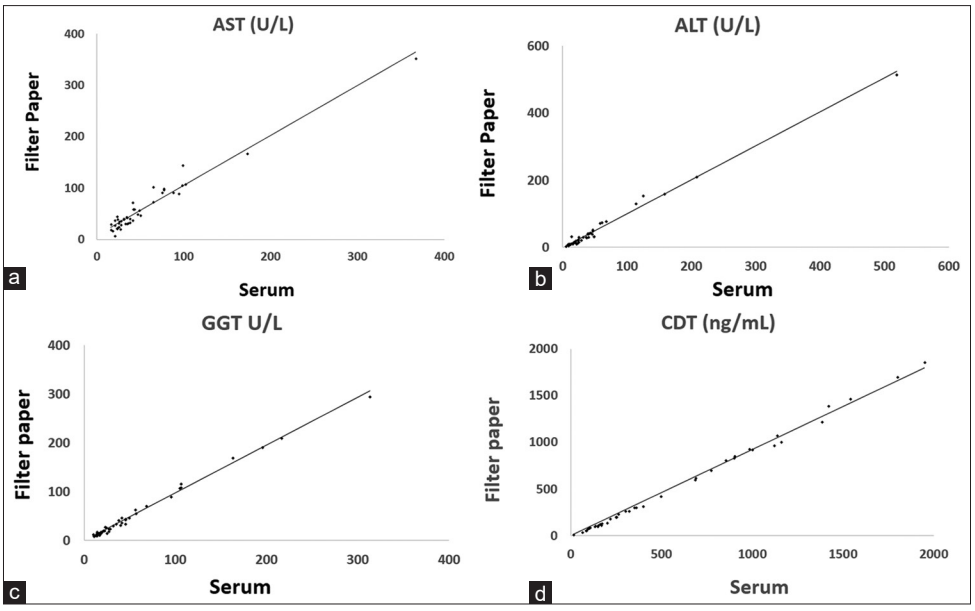


**Figure 1:** (a-d) Relationship in paired samples ($n$ = 45): Regression curve for alcohol biomarker levels obtained from filter paper as compared with levels in direct serum. Each Individual values represent the mean of duplicate samples. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma gutamyl transferase, CDT: Carbohydrate deficient transferrin

The clinical profile of the subjects indicated alcohol use in a majority (85%) of the opioid dependent patients either on a daily or weekly basis. Previous literature had reported diverse rates of alcohol use in patients on agonist maintenance, mostly methadone.[6] However, there is very limited literature on the rates of alcohol use among buprenorphine-maintained patients,[23] especially from India.[8] Tobacco use was observed among all the subjects included in the study, while cannabis use was found in 45% of patients. Alcohol and tobacco are the substances most commonly used together, because of their shared neurobiological mechanisms augmenting each other's rewarding effects.[24] Previous literature had reported cannabis use among the opioid-dependent population in Indian settings.[25] While interpreting the results in treatment settings, caution should be maintained as the effect of concurrent tobacco and heavy alcohol use may alter liver enzymes.[26] However, cannabis use *per se* has no effect on liver function.[27]

Buprenorphine is one of the most widely used opioid agonist medications in India. Compliance to buprenorphine, without any side effect, among more than 90% of the study patients is in accordance with recent reports.[28] The clinical profile of the patients showed 34% of subjects with moderate ASSIST scores and the remaining 11% with high-risk scores. These results indicate the importance of an objective screening of these patients for harmful alcohol use on a regular basis.

At the same time, the study has a few limitations. The study was planned in a routine clinical setting, and therefore, more recent and specific alcohol biomarkers were not assessed. The liver enzymes assessed for alcohol use have limitations in terms of sensitivity and specificity. The number of samples included was determined on the feasibility of sample collection from a community setting on a pilot basis.

However, the results of the study can be applied to any drug treatment community setting. This study advocates the need for routine use of objective methods to corroborate with patients' self-report during a clinical judgment. Future studies comprising more specific alcohol biomarkers, from multiple settings, are warranted.

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### Conflicts of interest
There are no conflicts of interest.

## REFERENCES

1. World Drug Report, 2017. United Nations Office on Drugs and Crime, World Drug Report 2017 (United Nations publication). Available from: https://www.unodc.org/wdr2017/field/Booklet_1_EXSUM.pd. [Last accessed on 2019 Mar 06].
2. Ray R, Chopra A. Monitoring of substance abuse in

India – Initiatives & experiences. Indian J Med Res 2012;135:806-8.
3. Ambekar A, Agrawal A, Rao R, Mishra AK, Khandelwal SK, Chadda RK. Magnitude of Substance Use in India. New Delhi: Ministry of Social Justice and Empowerment, Government of India; 2019.
4. Mattick RP, Breen C, Kimber J, Davoli M. Buprenorphine maintenance versus placebo or methadone maintenance for opioid dependence. Cochrane Database Syst Rev 2014;6:CD002207.
5. Stenbacka M, Beck O, Leifman A, Romelsjö A, Helander A. Problem drinking in relation to treatment outcome among opiate addicts in methadone maintenance treatment. Drug Alcohol Rev 2007;26:55-63.
6. Hillebrand J, Marsden J, Finch E, Strang J. Excessive alcohol consumption and drinking expectations among clients in methadone maintenance. J Subst Abuse Treat 2001;21:155-60.
7. Soyka M. Alcohol use disorders in opioid maintenance therapy: Prevalence, clinical correlates and treatment. Eur Addict Res 2015;21:78-87.
8. Varshney M, Ambekar A, Lal R, Yadav D, Rao R, Mishra A. Brief interventions for harmful alcohol use in opioid-dependent patients on maintenance treatment with buprenorphine: A prospective study from India. Addict Disord Their Treat 2016;15:129-35.
9. Peterson K. Biomarkers for alcohol use and abuse--A summary. Alcohol Res Health 2004-2005;28:30-7.
10. Kalapatapu RK, Chambers R. Novel objective biomarkers of alcohol use: Potential diagnostic and treatment management tools in dual diagnosis care. J Dual Diagn 2009;5:57-82.
11. McDonald H, Borinskya S, Kiryanov N, Gil A, Helander A, Leon DA. Comparative performance of biomarkers of alcohol consumption in a population sample of working-aged men in Russia: The Izhevsk Family Study. Addict Abingdon Engl 2013;108:1579-89.
12. Hietala J, Koivisto H, Anttila P, Niemelä O. Comparison of the combined marker GGT–CDT and the conventional laboratory markers of alcohol abuse in heavy drinkers, moderate drinkers and abstainers. Alcohol Alcohol 2006;41:528-33.
13. Schito M, Peter TF, Cavanaugh S, Piatek AS, Young GJ, Alexander H, *et al*. Opportunities and challenges for cost-efficient implementation of new point-of-care diagnostics for HIV and tuberculosis. J Infect Dis 2012;205:S169-80.
14. Singh MP, Majumdar M, Budhathoki B, Goyal K, Chawla Y, Ratho RK. Assessment of dried blood samples as an alternative less invasive method for detection of Hepatitis E virus marker in an outbreak setting. J Med Virol 2014;86:713-9.
15. Sarkar S, Singh MP, Ratho RK. Dried blood spot for Ebola testing in developing countries. Lancet Infect Dis 2015;15:1005.
16. Quraishi R, Lakshmy R, Mukhopadhyay AK, Jailkhani BL. Transaminase extraction and stability from dried serum samples. Sch J App Sci 2014;2:169-70.
17. Quraishi R, Lakshmy R, Mukhopadhyay AK, Jailkhani BL. Creatinine measurement and stability in dried serum. J Diabetes Sci Technol 2012;6:988-9.
18. Reitman S, Frankel S. A colorimetric method for the determination of serum Glutamic oxaloacetic and Glutamic pyruvic transaminases. Am J Clin Path 1957;28:56-63.
19. Nandeesha H, Rajappa M, Kadhiravan T, Srilatha K, Harichandrakumar KT, Thyagarajan D. Carbohydrate deficient transferrin and interleukin-6 as predictors of fibrosis in alcohol cirrhosis. Ind J Clin Biochem 2016;31:117-20.
20. Humeniuk R, Ali R, WHO ASSIST Phase II Study Group. Validation of the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) and Pilot brief Intervention: A Technical Report of Phase II Findings of the WHO ASSIST Project. Geneva: World Health Organization; 2006.
21. Quraishi R, Singh D, Ambekar A, Jain R, Rao R. Gamma gutamyl transferase measurement in dried serum spots. Alcohol Clin Exp Res 2018;42:24A. Available from: https://onlinelibrary.wiley.com/doi/epdf/10.1111/acer.13833.[Last accessed on 2019 Mar 06].
22. Bertaso A, Sorio D, Vandoros A, De P, Elio F, Bortolotti F, *et al*. The use of finger-prick dried blood spots (fpDBS) and capillary electrophoresis for carbohydrate deficient transferrin (CDT) screening in forensic toxicology. Electrophoresis 2016;37:2867-74.
23. Klimas J, Muench J, Wiest K, Croff R, Rieckman T, McCarty D. Alcohol screening among opioid agonist patients in a primary care clinic and an opioid treatment program. J Psychoactive Drugs 2015;47:65-70.
24. Adam S. Psychopharmacology of tobacco and alcohol comorbidity: A review of current evidence. Curr Addict Rep 2017;4:25-34.
25. Bagra I, Krishnan V, Rao R, Agrawal A. Does cannabis use influence opioid outcomes and quality of life among buprenorphine maintained patients? A cross-sectional, comparative study. J Addict Med 2018;12:315-20.
26. Wannsmethee SG, Shaper AG. Cigarette smoking and serum liver enzymes: The role of alcohol and inflammation. Ann Clin Biochem 2010;47:321-6.
27. Adejemo AC, Ajayi TO, Adegbala OM, Adejumo KL, Alliu S, Akinjero AM, *et al*. Cannabis use is associated with reduced prevalence of progressive stages of alcoholic liver disease. Liver Int 2016;38:1475-86.
28. Ambekar A, Rao R, Agrawal A, Kathiresan P. Research on opioid substitution therapy in India: A brief, narrative review. Indian J Psychiatry 2018;60:265-70.