



RESEARCH PAPER

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Prevalence and comparative analysis of HBs, HCV, CRP, T.B and H. pylori in drug addicted individuals from local population of Charsadda, Khyber Pakhtunkhwa, Pakistan

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Abstract

The main objective of the current study was to evaluate the prevalence of infectious diseases in drug addicts compared to normal individuals. The study was conducted in Biotechnology Department Bacha Khan University Charsadda, KPK, Pakistan. Total 80 drug addict's blood samples including smoker, alcoholic, cocaine and cannabis users (20 of each) were collected from age range 25-50 years from local area in EDTA tube and multiple diagnostic assay were performed. Results indicated that total of 22.5% HBs, 50% HCV, 17.5% CRP, 18.5% T.B and 65% H. pylori prevalence was found in each drug category. The results suggested an interaction on the additive scale between drugs use and HBV infection, and an interaction on the multiplicative scale with HCV infection. Additional studies are required to investigate the possible biological role of drugs in the development of infectious forms of HBs, HCV, CRP, T.B and H. Pylori and effect on expression of gene and DNA of addicted individuals. There is dire need of awareness in public about the lethal effects of use of these addictive drugs.

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Introduction

Drugs are substances, causing delusional feelings by altering the perception of reality including both biological and psychological dependence (Raviglione, Snider Jr *et al.*, 1995). Consumption of drugs containing materials imposes drastic effects on human health such as tobacco, alcohol consumption, cocaine, Cannabis use etc. Cigarette smoke and tobacco contains thousands of dangerous chemicals. Eleven compounds (2-naphthyleamine, 4-aminobiphenyle, benzene, vinyl chloride, ethylene oxide, arsenic, beryllium, nickel compounds, chromium, cadmium and polonium-210), classified as IARC group 1 human carcinogens have been reported in tobacco smoke (Hoffmann, Brunnemann *et al.* 1975, Gray and Roberts 1988).

Cigarette smoking causes several diseases such as cardiovascular disease (CVD), Lung cancer and chronic obstructive pulmonary disease (Das 2003, Alberg, Brock *et al.*, 2013). Cigarette smoke provides easy entry to mycobacterium tuberculosis, is the leading cause of the pathogenesis of TB (Shaler, Horvath *et al.*, 2013). A mathematical survey estimated that from 2010 to 2050 about 18 million more TB cases and 40 million more TB deaths will occur as result of cigarette smoking (Basu, Stuckler *et al.*, 2011). Smoking enhances inflammation which is manifested by raised plasma levels of inflammatory markers like C- reactive protein (CRP) and white blood cell (WBC) counts (Libby, Ridker *et al.*, 2002, Lau, Dhillon *et al.*, 2005). High basal levels of CRP increase the risk of developing obesity, diabetes, hypertension and CVD (Lopez-Garcia, Schulze *et al.*, 2005). *Helicobacter pylorus* is a human pathogen that colonizes the gastric mucosa and causes permanent gastric inflammation (Rosenstock, Kay *et al.*, 1997). The use of tobacco impairs the immune system and is an important factor in peptic ulcer disease and dyspeptic symptoms (Holt 1988).

Cannabis is also known as marijuana, a complex plant, with major compounds such as delta-9-tetrahydrocannabinol and cannabidiol, which have strong addictive effect (Degenhardt, Coffey *et al.* 2010).

Marijuana smoking is common and believed to relieve many symptoms but continuous use lead to liver fibrosis (Brunet, Moodie *et al.* 2013). Acute effects of cannabis include increased heart rate along with an increased blood pressure and then decreased vascular resistance-induced orthostatic hypotension (Greydanus, Hawver *et al.*, 2013).

Another powerful drug is Cocaine, an addictive stimulant that directly affects the brain. Some of the severe side effects of cocaine consumption are vasoocclusive events such as myocardial infarction and stroke, but apart from that, cocaine could affect red blood cells (RBCs) and alter the rheological behavior of blood and increases blood viscosity (Cagienard, Schulzki *et al.*, 2013). Frequent users of cocaine are likely to have multiple risk factors for active pulmonary TB causes symptoms like confined air sacks, intensive coughing and other acute pulmonary complications of cocaine inhalation promotes transmission of TB (Pablos-Méndez, Knirsch *et al.*, 1997). The exact pathophysiology of cocaine-induced gastro duodenal injury is unclear. Cocaine over stimulates the sympathetic nervous system, causing vasoconstriction-Induced ischemia and necrosis of the mucosal wall (Tiwari, Moghal *et al.*, 2006).

An alcohol is any organic compound in which the hydroxyl functional group (–OH) is bound to a saturated carbon atom. The liver is particularly susceptible to alcohol-related injury because it is the primary site of alcohol metabolism. As alcohol is broken down in the liver, a number of potentially dangerous by-products are generated, such as acetaldehyde and highly reactive molecules called free radicals. Perhaps more so than alcohol itself, these products contribute to alcohol-induced liver damage (Mezey, Kolman *et al.*, 1988). Association between liver disease and heavy alcohol consumption was recognized more than 200 years ago (Smart and Mann 1992).

Alcohol-consuming hosts are considered "Immuno-Compromised" because the incidence and severity of infectious diseases among them are greater than for abstainers (Szabo, Mandrekar *et al.*, 1995). Individuals with alcohol dependence are particularly susceptible to lung infections such as TB and pneumonia (Happel, Dubin *et al.*, 2005).

Alcohol has been shown to reduce macrophage response to immune system modifiers (e.g. cytokines, including interleukin-6 (IL-6), IL-1 β , TNF- α , and IL-8) and to prevent the protective effect exerted by the cytokines (Neuman 2003). Probably more than half of the world population is infected with the spiral gastric bacterium *H. pylori*. Infection with *H. pylori* is the main cause of chronic gastritis and a major risk factor of peptic ulcer and gastric cancer. Alcohol has strong antimicrobial activity and stimulates gastric acid secretion. Alcohol consumption may therefore compromise the living conditions of *Helicobacter pylori* in the stomach (Goodwin, Mendall *et al.*, 1997). The aim of current study is to evaluate the prevalence and comparative analysis of Hepatitis B surface antigens (HBS), Hepatitis C virus (HCV), C- reactive protein (CRP), T.B and *H. pylori* in cigarette smokers, cocaine users, cannabis users and alcohol addicted individuals.

Materials and methods

Sample collection and Serum isolation

Blood samples were collected from addicted person (mean age ranges from 25-50 years) identified from both local population and foundation. Total 5ml blood was taken from each individual in two separate EDTA tubes and was processed at Department of Biotechnology, Bacha Khan University Charsadda and was stored freezer for further analysis. In order to isolate serum from blood samples, 2ml blood was transferred to Eppendorf tubes and were centrifuged at 2000rpm for 2 min. The supernatant was transferred through micropipette in a separate tube and stored for further analysis.

Diagnostic Tests

Reagents Preparation

All the reagents (for HBs, HCV, CRP, T.B, *H. pylori*) and samples were allowed to equilibrate at room temperature [18-30°C] for at least 15-30 min.

ELISA for HBs and HCV

The ELISA wells were marked as three for negative, two for positive controls and one blank neither samples nor HRP- conjugate was added into the blank well.

Total of 50 μ l of each of positive control, negative control, and specimen, were added into their respective wells along with the addition of 50 μ l of HRP conjugate in each well except blank. ELISA plate was incubated for 60 min at 30°C and washed 5 times with diluted buffer. 50 μ l of Chromogen A and 50 μ l of Chromogen B solutions were added into each well including the blank incubated at 37°C for 15 min in dark. The enzymatic reaction between the Chromogen solutions and HRP –conjugate produced a blue color in each positive control and positive sample wells. The results were determined by using a dual wavelength plate reader.

CRP Latex Test

The reagent was shaken gently to disperse the particles. A drop of undiluted serum was placed on to the circle of the test slide using the disposable pipettes. One drop of the latex reagent was added next to the drop of serum and was used to spread the reagent and serum sample over the entire area of the test circle. At the end test slide was rinsed with distilled water.

Tuberculosis (TB) Test

T.B IgG/IgM rapid test was performed by using T.B strip and serum. Total of 2-3 drops of sample serum (approximately 60-90 μ l) was transferred to the test strip and two drops of buffer were added. After few min the colored line was appeared and results were recorded. In the presence of C band, if only IgM band appears, it indicates the presence of anti-TB IgM and the sample is IgM positive. In the presence of C band, if only IgG band appears, it indicates the presence of anti-IgG and sample is IgG positive. In the presence of C band, if both IgM and IgG band appears, it indicates the anti-TB IgG and IgM presence and the sample is IgG and IgM positive. The appearance of only C band with burgundy colour indicated absence of the antibodies in the samples.

H. pylori Test

The *H. pylori* Ab rapid test was performed using serum. One drop of serum (approximately 30 μ l) was transferred on the test strips and one drop of buffer (approximately 40 μ l) was added. After few min colored line was appeared and results were recorded.

Appearance of two distinct red lines, one line in the control region (C) and second in test region (T), showed positive results. Only one red line appearance in the control region (C) not in test region indicated negative results. The red color concentration in the test line region (T) varies depending on the conception of *H. pylori* antibodies in the specimen. Therefore, any shade of red in the test region (T) was considered positive.

Results

Cigarette Smokers

Serum was isolated from blood samples of 20 cigarette smokers and performed different diagnostic assay to determine the presence of HBs, HCV, CRP, T.B and *H. pylori*. Out of 20 samples only six samples (30%) were found positive for the HBs antigen, four samples (20%) were found positive for HCV virus and only three samples (15%) were found positive for CRP. In the case of T.B, 3 (15%) and for *H. pylori* 13 (65%) of the total samples were found positive. Furthermore, 10 samples were collected randomly from general population and all were found negative.

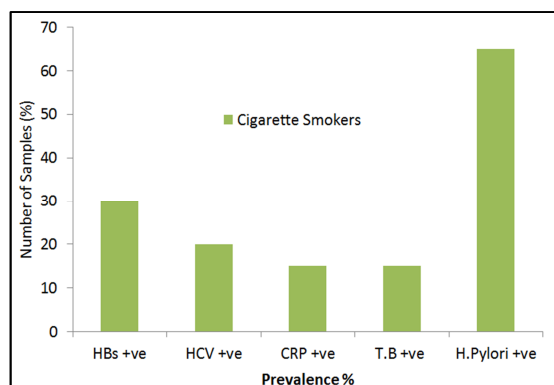


Fig. 1. Prevalence (%) of HBs, HCV, CRP, T.B and *H. pylori* in cigarette smokers.

Cannabis Users

Serum samples were isolated from 20 cannabis users for diagnostic purposes. None of the samples were found positive for HBs antigen in cannabis users as the prevalence percentage of HBs was considered 0%. 14 samples (70%) were found positive for the HCV whereas only 3 samples (15%) were found positive for CRP while the rest of them were found negative. Quite similar results were obtained in case of T.B and

H. pylori. Prevalence of T.B and *H. pylori* in cannabis users was observed 0% and 60% respectively as shown in (Fig. 2).

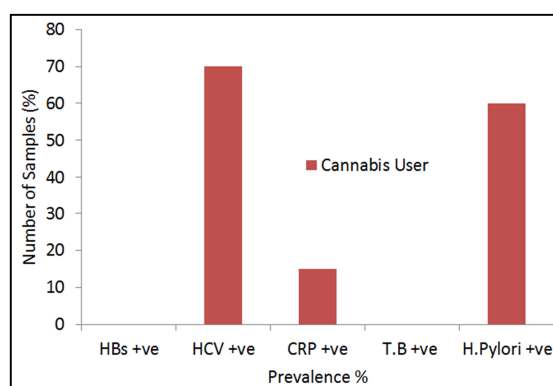


Fig. 2. Prevalence (%) of HBs, HCV, CRP, T.B and *H. pylori* in cannabis users.

Cocaine Users

Fig. 3 indicated the results obtained after analysis of serum samples of 20 different cocaine users. 4 (20%) samples were found positive for HBs antigen, 12 (60%) samples were HCV positive and 8 (40%) samples were positive in case of CRP. According to statistical analysis on the prevalence of T.B and *H. pylori*, high prevalence was observed for *H. pylori* i.e 65% whereas T.B counted for 30% prevalence of total samples.

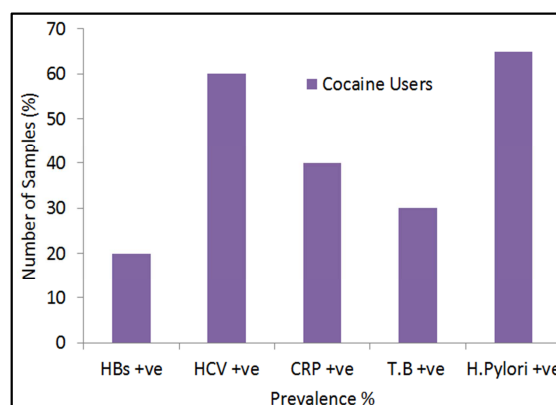


Fig. 3. Prevalence (%) of HBs, HCV, CRP, T.B and *H. pylori* in cocaine users.

Alcohol Drinkers

Prevalence of HBs, HCV, CRP, T.B and *H. pylori* was determined using serum from 20 alcohol drinkers from general population. HBs antigen, HCV and CRP were 40 %, 50 % and 0 % respectively.

Highest H. pylori prevalence (70%) was observed in alcohol drinkers compared to the prevalence of T.B (30%) in same group as shown in (Fig. 4).

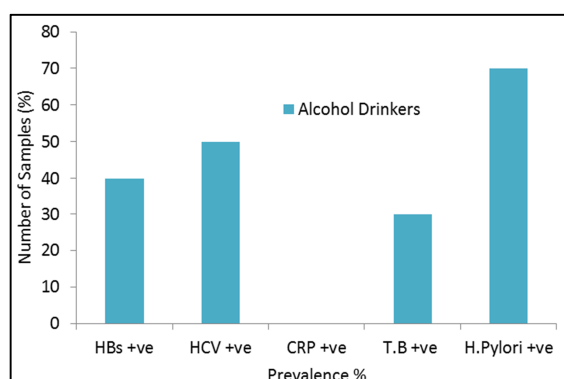


Fig. 4. Prevalence (%) of HBs, HCV, CRP, T.B and H. pylori in alcohol drinkers.

Comparative Analysis

From comparative analysis of HBs + ve in cigarette smokers, cannabis users, cocaine users, and alcohol drinkers, it was observed that HBs + ve prevalence was highest in alcohol drinkers (40%) followed by cigarette smokers (30%) and lowest in cocaine users (20%), while there was no HBs + ve found in cannabis users. HCV + ve prevalence was highest in cannabis users (70%) followed by cocaine users (60%) followed by alcohol drinkers (50%) and lowest in cigarette smokers (20%). CRP + ve prevalence was found highest in cocaine users (40%), in cannabis users (15%) and cigarette smokers (15%), whereas 0% in alcohol drinkers. T.B + ve prevalence was highest in cocaine and alcohol users (30%) followed by cigarette smokers (15%) and there was no T.B + ve found in cannabis users. H. pylori + ve prevalence was highest in alcohol drinkers (70%) followed by cocaine users and cigarette smokers (65%) followed by cannabis users (60%) shown in (Fig. 5, Table No. 1).

HBs (+ve) Prevalence: Alcohol Drinkers > Cigarette Smokers > Cocaine Users > Cannabis Users

HCV (+ve) Prevalence: Cannabis Users > Cocaine Users > Alcohol Drinkers > Cigarette Smokers

CRP (+ve) Prevalence: Cocaine Users > Cannabis Users = Cigarette Smokers > Alcohol Drinkers

T.B (+ve) Prevalence: Cocaine Users = Alcohol Drinkers > Cigarette Smokers > Cannabis Users

H. pylori (+ve) Prevalence: Alcohol Drinkers > Cocaine Users = Cigarette Smokers > Cannabis Users

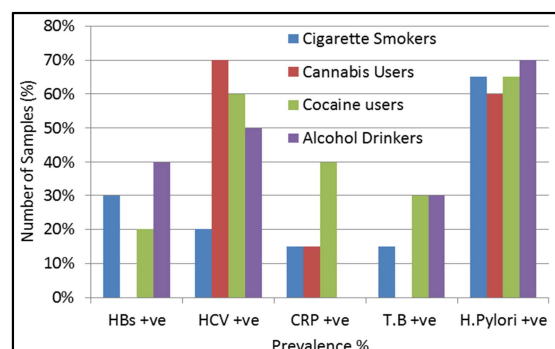


Fig. 5. Comparative Analysis of Prevalence (%) of HBs, HCV, CRP, T.B and H. pylori.

Discussion

The current study included 80 drug users which have 22.5% HBs, 50% HCV, 17.5% CRP, 18.5% T.B and 65% H. pylori prevalence (Table 1). The results suggested an interaction on the additive scale between drugs use and HBV infection, and an interaction on the multiplicative scale with HCV infection. A cohort study conducted in southern Taiwan showed that drug users had higher prevalence of HBs and HCV infection (Wang, Chang *et al.*, 2002). Study conducted by (Ghasemian *et al.*, 2009) found that T.B prevalence was 22 % in cigarette smokers which is high as compared to our findings (Ghasemian, Najafi *et al.*, 2009). Similarly a study conducted by (Ahmed *et al.*, 2010) found that T.B prevalence was 40% in cigarette smokers which is also higher as compared to our study which indicated that the reason may be due to increase resistance of our population to the T.B infection/Bacterial pathogens (Awaisu, Mohamed *et al.*, 2010). The prevalence percentage of H. pylori in cigarette smokers in our findings was 65% (Moy, Fan *et al.*, 2010) and (Moy *et al.*, 2007) concluded that smokers experienced 80% increased risk of gastric cancer (Kim, Lee *et al.*, 2007). Study carried out by (Konturek *et al.*, 2003) concluded that out of 5967 patients, 31.8% were ulcerated; 9.2% had gastric, 17.2% duodenal and 5.4% both gastric and duodenal ulcers (Konturek, Bielański *et al.*, 2003). H. pylori were found in 72.5% of gastric ulcer patients, in 83.6% of duodenal ulcer patients, in 76.9% of gastro duodenal ulcer patients and in 64.8% of dyspeptic patients. Our results are nearly similar to the results reported.

Table 1. Comparative Analysis of Prevalence (%) of HBs, HCV, CRP, T.B and H. pylori.

Category	Total Samples	Cigarette Smokers	Cannabis Users	Cocaine users	Alcohol Drinkers
HBs +ve	20	30%	0%	20%	40%
HCV +ve	20	20%	70%	60%	50%
CRP +ve	20	15%	15%	40%	0%
T.B +ve	20	15%	0%	30%	30%
H.Pylori +ve	20	65%	60%	65%	70%

The most widely consumed drug in the world is cannabis, cocaine and tobacco (Kruse, Barbour *et al.* 2009). The majority of drug users are associated with prevalence of HBs, HCV and HIV infection. A study carried out in turkey indicated that drug usage is the highest risk factor for acquisition of HBV, HCV and HIV infections (Keramat, Eini *et al.*, 2011). Turkey is considered as a region with intermediate endemicity for HBV (2-7%) and low endemicity for HCV (< 2%) (ÖNER, YAPICI *et al.* 2011), comparatively having low prevalence of HBs and HCV to current study which is HBs (22.5%) and HCV (50%).

The current study found a low rate of HBs Ag positivity (2.6%) in drug addicts, whereas the rate of anti-HCV was high (9.4%). (Yenen *et al.*, 1993) reported that the prevalence of HBs Ag and anti-HCV among drug addicts in Istanbul, Turkey, was 7.3% and 54.8%, respectively, which were considerably lower prevalence of HBs and nearly similar prevalence of HCV as compared to our study (Yenen, Beyazyürek *et al.* 1993). The reason for their low prevalence is that in Turkey, vaccination against HBV began in the early 1990s (Ozer, Yakupogullari *et al.*, 2011) and from the history of these addicted groups in our study, no proper vaccination against HBV has been reported. Similarly, study carried out by (Mathei *et al.*, 2004) showed a relatively low rate of HCV prevalence of 24.5% in Antwerp and 12.5% in Limburg (MatheI, Robaeys *et al.*, 2004). Prevalence of HCV in Asia ranges from 2.15-3.9% (Zidan, Scheuerlein *et al.*, 2012). China is the only country in Asia that was classified as high endemic area with prevalence of 7-20% for HBV infection which is almost same to our current result. The reason for their low prevalence is geographic location immunity differences and environmental factors.

A study conducted by (O'Loughlin *et al.*, 2008) showed that CRP levels were higher in youth with tobacco, alcohol, cannabis use, and with cocaine dependence (O'Loughlin, Lambert *et al.*, 2008). Thus the data presented here is consistent with earlier work showing that higher levels of CRP are associated with drug use. A study of smoking and inflammatory markers in 2,920 British men showed that current smokers had higher levels of CRP than never-smokers (Wannamethee, Lowe *et al.*, 2005), and a cross-sectional study of 2,999 Chinese men found that CRP increased across never, former and current smokers (Lao, Jiang *et al.* 2009). Study conducted by (Furie *et al.*, 2000) also has linked elevated CRP levels to smoking tobacco and drug users (Furie, Raffanella *et al.*, 2000). In the case of cannabis, a recent analysis of the NHANES data (Rajavashisth, Shaheen *et al.* 2012) found that the prevalence of elevated C reactive protein (>0.5mg/dl) was significantly less among past or current users than among non-marijuana users. The associations between drugs users and CRP were positive and there was no sign of an anti-inflammatory effect, as some reports have suggested (Kelley and Dantzer 2011). As expected, higher CRP levels were associated with several inter-correlated indicators of lower socio-economic status (poverty, less parental education, lower parental occupational status) as well as of poor health. The many significant associations with CRP levels seen in the cross-sectional data underline the difficulty of distinguishing between causal pathways and correlations among risk factors.

The prevalence percentage of H. pylori in alcohol users in our findings is 70%. Similarly another study by (Li Zhang *et al.* 2009) showed that H. pylori infection was positive in 27.3% of patients (Zhang, Wang *et al.* 2009). In Japan, seropositivity for H. pylori IgG antibody was 79.3% (Shinchi, Ishii *et al.*, 1997).

From previous studies, it was found that alcohol usage, smoking and other addicted drugs usage are risk factors for *H. pylori* infection which leads to gastric perforations and ultimately gastric cancer.

Concluding Remarks

All the addictive drugs are very dangerous for health and causes several diseases such as cardiovascular disease (CVD) and pulmonary disease. They enhance inflammation, rises plasma levels of inflammatory markers like C- reactive protein (CRP) and white blood cell (WBC) counts. The main objective of present study was to find the effect of these harmful drugs on human health. Furthermore, additional studies are required to investigate the possible biological role of drugs in the development of infectious forms of HBs, HCV, CRP, T.B and *H. pylori*. Also it is recommended that further research should be conducted to check out the effects of these addictive drugs on the expression of genes in the addictive individuals.

There is dire need of awareness in public about the lethal effects with use of these addictive drugs.

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Declaration of interest

None of the authors of this paper had any personal or financial conflicts of interest.

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