INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 4, p. 443-451, 2018

RESEARCH PAPER OPEN ACCESS

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

Hasnain Jan[1], Ayesha Naeem[1], Sajjad Ali Shah[1], Wali Muhammad[*2], Muhammad Asad Ullah[2], Shah Faisal[1], Hazrat Usman[1], Ayyaz Khan[2], Sifatullah[1]

[1]*Department of Biotechnology, Bacha Khan University, Charsadda, KPK, Pakistan*

[2]*Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan*

Key words: Drug addicts, Hepatitis C Virus, Infectious diseases

http://dx.doi.org/10.12692/ijb/12.4.443-451 Article published on April 30, 2018

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.

* **Corresponding Author:** Wali Muhammad ✉ wali.biotech5511@gmail.com

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-napthyleamine, 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-1β, 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 50µl of each of positive control, negative control, and specimen, were added into their respective wells along with the addition of 50µl 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. 50µl of Chromogen A and 50µl 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 shacked 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 60-90µl) 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 30µl) was transferred on the test strips and one drop of buffer (approximately 40µl) 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 assey 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.

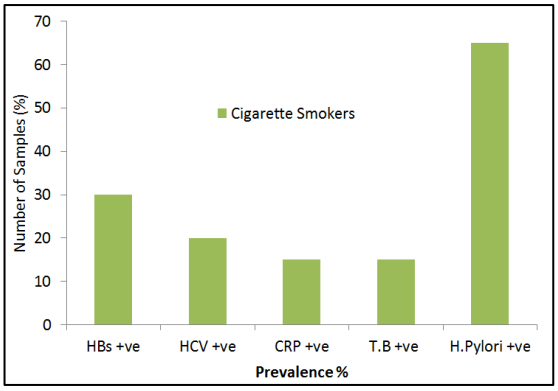

Fig. 1. Prevalence (%) of HBs, HCV, CRP, T.B and H. pylori in cigarette smokers.

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).

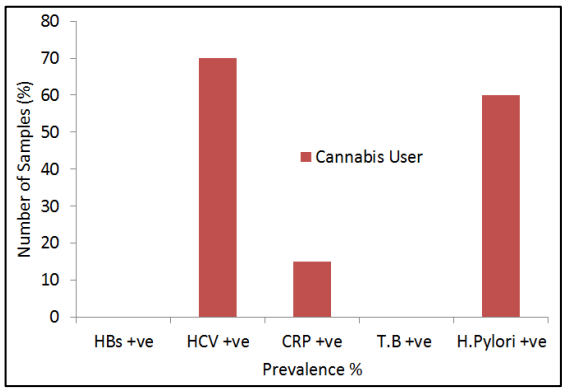

Fig. 2. Prevalence (%) of HBs, HCV, CRP, T.B and H. pylori in cannabis users.

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.

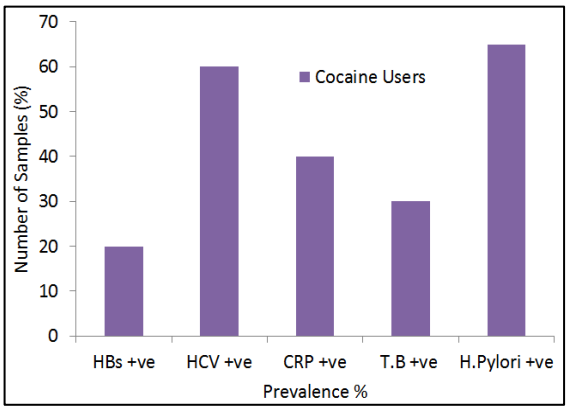

Fig. 3. Prevalence (%) of HBs, HCV, CRP, T.B and H. pylori in cocaine users.

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).

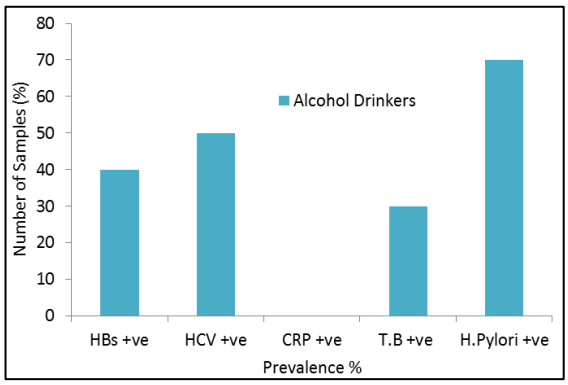

Fig. 4. Prevalence (%) of HBs, HCV, CRP, T.B and H. pylori in alcohol drinkers.

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 Uses > 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

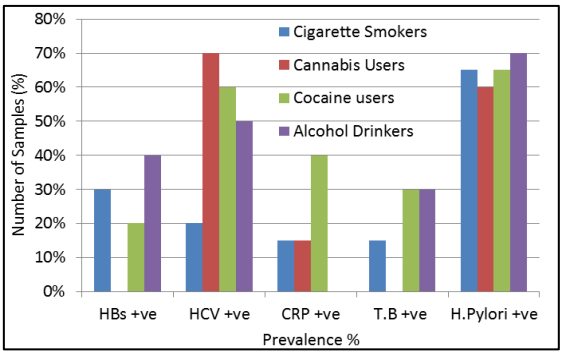

Fig. 5. Comparative Analysis of Prevalence (%) of HBs, HCV, CRP, T.B and H. pylori.

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, Raffanello *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.

Acknowledgments

The authors are thankful to Head of the Department of Biotechnology, Bacha Khan University for providing research facilities.

Declaration of interest

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

References

Alberg AJ, Brock MV, Ford JG, Samet JM, Spivack SD. 2013. "Epidemiology of lung cancer: Diagnosis and management of lung cancer: American College of Chest Physicians evidence-based clinical practice guidelines." Chest **143(5),** e1S-e29S.

Awaisu AMHN, Mohamed NA Aziz SAS, Sulaiman NM, Noordin AR, Muttalif A, Mahayiddin A. 2010. "Tobacco use prevalence, knowledge, and attitudes among newly diagnosed tuberculosis patients in Penang State and Wilayah Persekutuan Kuala Lumpur, Malaysia." Tobacco induced diseases **8(1),** 3.

Basu S, Stuckler D, Bitton A, Glantz SA. 2011. "Projected effects of tobacco smoking on worldwide tuberculosis control: Mathematical Modelling Analysis." Bmj **343,** d5506.

Brunet LEE, Moodie K, Rollet C, Cooper S, Walmsley M, Potter MB, Klein CC, Investigators IC. 2013. "Marijuana smoking does not accelerate progression of liver disease in HIV–hepatitis C coinfection: A longitudinal cohort analysis." Clinical Infectious Diseases **57(5),** 663-670.

Cagienard F, Schulzki T, Furlong P, Reinhart W. 2013. "Cocaine induces a reversible stomatocytosis of red blood cells and increases blood viscosity." Clinical Hemorheology and Microcirculation **55(3),** 321-329.

Das SK. 2003. "Harmful health effects of cigarette smoking." Molecular and Cellular Biochemistry **253(1-2),** 159-165.

Degenhardt L, Coffey C, Carlin JB, Swift W, Moore E, Patton GC. 2010. "Outcomes of occasional cannabis use in adolescence: 10-year follow-up study in Victoria, Australia." The British Journal of Psychiatry **196(4),** 290-295.

Furie MB, Raffanello JA, Gergel EI, Lisinski TJ, Horb LD. 2000. "Extracts of smokeless tobacco induce pro-inflammatory changes in cultured human vascular endothelial cells." Immunopharmacology **47(1),** 13-23.

Ghasemian R, Najafi N, Yadegarinia D, Alian S. 2009. "Association between cigarette smoking and pulmonary tuberculosis in men: A case-control study in Mazandaran, Iran." Archives of Clinical Infectious Diseases **4(3),** 135-141.

Goodwin CS, Mendall MM, Northfield TC. 1997. "Helicobacter pylori infection." The Lancet **349(9047),** 265-269.

Gray B, Roberts M. 1988. Analysis of chemical kinetic systems over the entire parameter space-I. The Sal'nikov thermokinetic oscillator. Proc. R. Soc. Lond. A, The Royal Society.

Greydanus D, Hawver EK, Greydanus MM, Merrick J. 2013. "Marijuana: current concepts." Frontiers in public health **1,** 42.

Happel KI, Dubin PJ, Zheng M, Ghilardi N, Lockhart C, Quinton LJ, Odden AR, Shellito JE, Bagby GJ, Nelson S. 2005. "Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae." Journal of Experimental Medicine **202(6),** 761-769.

Hoffmann D, Brunnemann K, Gori G, Wynder E. 1975. On the carcinogenicity of marijuana smoke. Recent advances in phytochemistry, Springer 63-81.

Holt P. 1988. "Environmental pollutants as co-factors in IgE production." Current opinion in immunology **1(4),** 643-646.

Kelley KW, Dantzer R. 2011. "Alcoholism and inflammation: neuroimmunology of behavioral and mood disorders." Brain, Behavior and Immunity **25,** S13-S20.

Keramat F, Eini P, Majzoobi M. 2011. "Seroprevalence of HIV, HBV and HCV in persons referred to hamadan behavioral counseling center, west of Iran." Iranian Red Crescent Medical Journal **13(1),** 42.

Kim JJ, Lee JH, Jung H-Y, Lee GH, Cho JY, Ryu CB, Chun HJ, Park JJ, Lee WS, Kim HS. 2007. "EMR for early gastric cancer in Korea: A multicenter retrospective study." Gastrointestinal Endoscopy **66(4),** 693-700.

Konturek S, Bielański W, Płonka M, Pawlik T, Pepera J, Konturek P, Czarnecki J, Penar A, Jedrychowski W. 2003. "Helicobacter pylori, non-steroidal anti-inflammatory drugs and smoking in risk pattern of gastroduodenal ulcers." Scandinavian Journal of Gastroenterology **38(9),** 923-930.

Kruse GR, Barbour R, Heimer R, Shaboltas AV, Toussova OV, Hoffman IF, Kozlov AP. 2009. "Drug choice, spatial distribution, HIV risk, and HIV prevalence among injection drug users in St. Petersburg, Russia." Harm Reduction Journal **6(1),** 22.

Lao XQ, Jiang CQ, Zhang WS, Adab P, Lam TH, Cheng KK, Thomas GN. 2009. "Smoking, smoking cessation and inflammatory markers in older Chinese men: The Guangzhou Biobank Cohort Study." Atherosclerosis **203(1),** 304-310.

Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S. 2005. "Adipokines: molecular links between obesity and atheroslcerosis." American Journal of Physiology-Heart and Circulatory Physiology **288(5),** H2031-H2041.

Libby P, Ridker PM, Maseri A. 2002. "Inflammation and atherosclerosis." Circulation **105(9),** 1135-1143.

Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, Willett WC, Hu FB. 2005. "Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction." The Journal of nutrition **135(3),** 562-566.

MatheI C, Robaeys G, Van Ranst M, Van Damme P, Buntinx F. 2004. "Molecular epidemiology of hepatitis C among drug users, correlation with clinical parameters, sexual behaviour and drug-related risk behaviour." Clinical Microbiology & Infection Supplement **10,** 444.

Mezey E, Kolman C, Diehl A, Mitchell M, Herlong H. 1988. "Alcohol and dietary intake in the development of chronic pancreatitis and liver disease in alcoholism." The American Journal of Clinical Nutrition **48(1),** 148-151.

Moy KA, Fan Y, Wang R, Gao Y-T, Mimi CY, Yuan J-M. 2010. "Alcohol and tobacco use in relation to gastric cancer: a prospective study of men in Shanghai, China." Cancer Epidemiology and Prevention Biomarkers 1055-9965. EPI-1010-0362.
Neuman MG. 2003. "Cytokines-central factors in alcoholic liver disease." Alcohol Research and Health **27,** 307-316.

O'Loughlin J, Lambert M, Karp I, McGrath J, Gray-Donald K, Barnett TA, Delvin EE, Levy E, Paradis G. 2008. "Association between cigarette smoking and C-reactive protein in a representative, population-based sample of adolescents." Nicotine & Tobacco Research **10(3),** 525-532.

Öner S, Yapici G, Şaşmaz CT, Kurt AÖ, Buğdayci R. 2011. "Hepatitis B, hepatitis C, HIV, and VDRL seroprevalence of blood donors in Mersin, Turkey." Turkish Journal of Medical Sciences **41(2),** 335-341.

Ozer A, akupogullari YY, Beytur A, Beytur L, Koroglu M. 2011. "Risk factors of hepatitis B virus infection in Turkey: A population-based, case-control study: Risk Factors for HBV Infection." Hepatitis Monthly **11(4),** 263.

Pablos-Méndez A, Knirsch C, Graham A, Barr MR, Lerner BH, Frieden TR. 1997. "Nonadherence in tuberculosis treatment: predictors and consequences in New York City." The American Journal of Medicine **102(2),** 164-170.

Rajavashisth TB, Shaheen M, Norris KC, Pan D, Sinha SK, Ortega J, Friedman TC. 2012. "Decreased prevalence of diabetes in marijuana users: cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) III." BMJ Open **2(1),** e000494.

Raviglione MC, Snider Jr D, Kochi A. 1995. "Global epidemiology of tuberculosis." Morbidity and mortality of a worldwide epidemic. JAMA **273(3),** 220-226.

Rosenstock S, Kay L, Rosenstock C, Andersen L, Bonnevie O, Jørgensen T. 1997. "Relation between Helicobacter pylori infection and gastrointestinal symptoms and syndromes." Gut **41(2),** 169-176.

Shaler CR, Horvath CN, McCormick S, Jeyanathan M, Khera A, Zganiacz A, Kasinska J, Stampfli MR, Xing Z. 2013. "Continuous and discontinuous cigarette smoke exposure differentially affects protective Th1 immunity against pulmonary tuberculosis." PLoS One **8(3),** e59185.

Shinchi K, Ishii H, Imanishi K, Kono S. 1997. "Relationship of cigarette smoking, alcohol use, and dietary habits with Helicobacter pylori infection in Japanese men." Scandinavian Journal of Gastroenterology **32(7),** 651-655.

Smart RG, Mann RE. 1992. "Alcohol and the epidemiology of liver cirrhosis." Alcohol Research and Health **16(3),** 217.

Szabo G, Mandrekar P, Catalano D. 1995. "Inhibition of superantigen-induced T cell proliferation and monocyte IL-1β, TNF-α, and IL-6 production by acute ethanol treatment." Journal of Leukocyte Biology **58(3),** 342-350.

Tiwari A, Moghal M, Meleagros L. 2006. Life threatening abdominal complications following cocaine abuse, SAGE Publications Sage UK: London, England.

Wang C-S, Chang T-T, Yao W-J, Chou P. 2002. "Comparison of hepatitis B virus and hepatitis C virus prevalence and risk factors in a community-based study." The American Journal of Tropical Medicine and Hygiene **66(4),** 389-393.

Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH. 2005. "Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease." European Heart Journal **26(17),** 1765-1773.

Yenen O, Beyazyürek M, Keskin K, Çavuşlu Ş, Sakarya S, Altunay H. 1993. "Prevalence of HBsAg, Anti-HBc, anti-HCV, anti-HTLV-1, anti-HIV-1/2 in intravenous drug abusers." Klimik Derg **6,** 35-36.

Zhang L-J, Wang S-Y, Huo X-H, Zhu Z-L, Chu J-K, Ma J-C, Cui D-S, Gu P, Zhao Z-R, Wang M-W. 2009. "Anti-Helicobacter pylori therapy followed by celecoxib on progression of gastric precancerous lesions." World Journal of Gastroenterology: WJG **15(22),** 2731.

Zidan A, Scheuerlein H, Schüle S, Settmacher U, Rauchfuss F. 2012. "Epidemiological pattern of hepatitis B and hepatitis C as etiological agents for hepatocellular carcinoma in iran and worldwide." Hepatitis Monthly **12(10 HCC).**