BIOLOGICAL MONITORING OF POLYCYCLIC AROMATIC HYDROCARBONS AS A POSSIBLE RISK FACTOR OF HEPATOCELLULAR CARCINOMA AMONG CASES OF CHRONIC ACTIVE HEPATITIS B AND C

By

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ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAHs) are among the most carcinogenic, mutagenic and toxic contaminants. Their exposure and metabolism to DNA-reactive metabolites in the body are considered to contribute to the etiology of many types of the human cancers.

Objectives: To find out if exposure to polycyclic aromatic hydrocarbons is a risk factor for development of hepatocellular carcinoma (HCC) among the exposed cases, to detect if the smoking is an augmented factor for development of hepatocellular carcinoma among exposed cases, and to find the effect of socio-demographic characteristics of cases of hepatocellular carcinoma exposed to hydrocarbons.

Subjects and Methods: A case control study was conducted between the period from the first of March 2015 to end of August 2017. The study was conducted in the outpatient clinic of the Department of Hepatology and Gastro-enterology at Theodor Bilharz Research Institute (TBRI). The minimum sample size required for the present study was calculated using Epi info program, considering following data: Two sided confidence level = 95%, power of test = 80%, ratio of control: cases = 1:1, percent of control exposed = 21%, percent of cases exposed = 42 %, and odds ratio = 2.8. Kelsey estimated number of cases = 77 and number of control = 77 subjects. All subjects of both groups were interviewed. Every patient was subjected to the selected interview sheet and biological monitoring of urinary 1-hydroxy pyrene as a biomarker for PAHs exposure.

Results: 73% of cases of HCC had increased level of 1-hydroxy pyrene in urine with statistical significance difference when compared to controls. There was a significant positive association between exposure to PAHs and development of HCC among case group (OR = 4.9). There was a significant association between smoking and abnormal high level of 1-hydroxy pyrene in urine (OR = 1.7) among the case group. There was a significant positive association between exposure to PAHs and development of HCC among males (OR = 1.6). There was neither statistical significance difference nor positive association between exposure to PAHs and development of HCC in urban areas (OR=0.8). There was a statistically significant positive association between exposure to PAHs and development of HCC among smoker (OR=1.7). There was neither statistical significance nor positive association between exposure to PAHs and development of HCC among patients with chronic active hepatitis C (OR=0.6). There was a highly positive correlation between 1-hydroxy pyrene and Alfa Feto Protein (AFP) among positive cases of 1-hydroxy pyrene in case group (OR=316.25).
Conclusion: Exposure to PAHs is considered as a risk factor of HCC among cases of hepatitis B and C. HCC cases had increased level of 1-hydroxy pyrene in urine with statistical significance difference when compared to controls. A significant positive association between exposure to PAHs and development of HCC among males and smokers were also detected.

Keywords: 1-hydroxy pyrene, HCC. Hepatitis B, Hepatitis C, PAHs.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are major pollutants in the environment formed during incomplete combustion of organic materials such as gasoline, diesel fuel, coal and oil. The substances are therefore found in heavily polluted air, water, soil and smoked food (WHO, 2013).

Polycyclic aromatic hydrocarbons (PAHs) are among the most carcinogenic, mutagenic and toxic contaminates. Their exposure and metabolism to DNA-reactive metabolites in the body are considered to contribute to the etiology of many types of the human cancers (EEAA, 2011).

Uptake of PAH in the body may be monitored by different biomarkers, for example metabolites in urine, urinary thioethers, urinary mutagenicity, PAH–protein adducts, and PAH–DNA adducts (Angerer and Schaller, 2009).

Hepatocellular carcinoma (HCC) is a major cause of cancer death worldwide. Its incidence is increasing, ranging between 3% and 9% annually depending on the geographical location, and variability in the incidence rates correspond closely to the prevalence and pattern of the primary etiologic factors (El-Zayadi et al., 2011).

Ezzat et al. (2014) stated that during the last 5-10 years, high incidence of HCC in Egypt which reaches about 21% in cirrhotic patients.

In a single center study over a decade in Egypt reported that, chronic infections with HBV or HCV have both been recognized as human liver carcinogens with a combined attributable fraction of at least 75% of all HCC cases (Hassan et al., 2011).

It was observed that the incidence of HCC all over the world is increasing year by year with no definite exploration for this problem. However, PAHs might be a risk factor especially among the cases of chronic active hepatitis B and C.

The present work aimed to find out if exposure to polycyclic aromatic hydrocarbons is a risk factor for development of hepatocellular carcinoma among the exposed cases, to detect if the smoking is an augmented factor for development of hepatocellular carcinoma among exposed cases, and to find the sociodemographic characteristics of cases of hepatocellular carcinoma.

SUBJECTS AND METHODS

This was a case control study which was conducted between the period from the first of March 2015 to end of August 2017. The study was conducted in the outpatient clinic of the Department of Hepatology and Gastro-entrology at Theodor Bilharz Research Institute (TBRI). The minimum sample size required for the present study was calculated using Epi info program, considering the following data: Two sided confidence level = 95%, power of test =
80%, ratio of control to cases = 1:1, percent of control exposed = 21% (El-Zayadi et al., 2011), percent of cases exposed = 42 %, and odds ratio = 2.8. Kelsey estimated number of cases = 77, and number of control = 77 subjects. The sample was selected from all cases registered at the place of the study and regularly followed up at the outpatient clinic. Cases were defined as patients with chronic active hepatitis B, C, or both with hepatocellular carcinoma (HCC). They were numbered and total was 120 patients. By using simple random technique, the cases were selected using table of random number to reach 77 cases. Controls were selected from all cases registered at the place of the study and regularly followed up at the outpatient clinic. Controls were defined as patients with chronic active hepatitis B, C, or both without hepatocellular carcinoma (HCC). They were numbered and total was 400 patients. By using simple random technique, the controls were selected using table of random number to reach 77 controls.

All the following data were collected from all subjects: Personal history, occupational history and medical history of chronic active hepatitis B and/or C and HCC. Clinical examination (general and local examination) and investigations included liver function tests, ultrasonography, triphasic CT, alpha fetoprotein and liver biopsy or fibro scan. Biological monitoring of 1-Hydroxy pyrene in urine was used as a biomarker of exposure to polycyclic aromatic hydrocarbons.

The preparatory phase took about six months from the first of March 2015 till the end of August 2015. During this phase, a review of literature was conducted in order to explain the risk of occupational and environmental exposure to polycyclic aromatic hydrocarbons and biological monitoring of them.

Written permission to implement the study was obtained from Ethic Committee of both Al-Azhar Faculty of Medicine and TBRI. Written permission to implement the study was also obtained from TBRI hospitals authority from the general manager of TBRI and Head of the Department of Hepatology and Gastroenterology at TBRI. Oral approval was taken from every subject before subjecting him to the interview sheet, urine sampling and clinical examination.

Pilot study was conducted to assess patient’s impression, reaction and cooperation with the study. The pilot study included 16 patients (8 subjects of case group and 8 subjects of control group). No modification of the interview sheet was conducted after the pilot study as respecting culture and time of the examined patients. So, the pilot sample was included in this study.

The implementation phase took about one year, from the first of September 2015 till the end of August 2016. During this phase, the researcher interviewed all included patients separately. Every patient was subjected to the selected parameters, and biological monitoring of urinary 1-hydroxy pyrene as a biomarker for PAHs exposure. It took about one and half hour for each subject.

The evaluation phase took about one year from the first of September 2016 till the end of August 2017.

Statistical analysis: The collected data were entered and analyzed using SPSS
package (version 17.0). Data were presented using frequencies and percentages. The level of 1-hydroxy pyrene in urine was measured and compared between case and control groups by socio-demographic factors using chi square test. P values ≤ 0.05 were used as indicators of statistical significance differences between the two studied groups. Odds ratio was also calculated for each studied factor.

RESULTS

The level of 1-hydroxy pyrene in urine was presented in Table 1. There was statistical significance difference between cases and controls with p < .0001 (73% of cases showed abnormal level and about 50% of them showed moderate elevation). It was shown that there was a positive association between exposure to PAHs and development of HCC (OR= 4.9–

Table (1): 1-Hydroxy pyrene in urine as a biomarker of exposure to Polycyclic Aromatic Hydrocarbons (PAHs) among the studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case group N = 77</th>
<th>Control group N = 77</th>
<th>Chi-square</th>
<th>Odds ratio (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-hydroxy pyrene in urine:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal level</td>
<td>21</td>
<td>50</td>
<td>21.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Abnormal level</td>
<td>56</td>
<td>27</td>
<td></td>
<td>4.9</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case group n = 56</th>
<th>Control group n = 27</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types of abnormalities:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High elevation</td>
<td>14</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Moderate elevation</td>
<td>28</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Low elevation</td>
<td>14</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Very low elevation</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>27</td>
<td>28.475</td>
</tr>
</tbody>
</table>

*Significant

The level of 1-hydroxy pyrene in urine in the studied cases and controls by their characteristics was presented in Table 2. There was a statistically significant difference between the two groups by their sex, residence, smoking habit, type of hepatitis, (p > 0.05). However, it was noticed that males had 1.65 fold more than females to develop HCC on top of chronic active hepatitis B and C when exposed to PAHs (OR = 1.65). A positive association between smoking and development of HCC on top of chronic active hepatitis B and C in case group when exposed to PAHs was also found with odds ratio of 1.7. A very high association, however, between the presence of 1-Hydroxy pyrene in urine and elevated Alfa Feto
protein (AFP) among the cases (OR = 316.25), and there was a statistically significant difference between the exposed and non-exposed group (p <0.001). On the other hand, however, a significant negative association was detected between 1-hydroxy pyrene and signs of decompensation (OR= 0.10) (Table 2).

Table (2): 1-hydroxy pyrene in urine among the studied groups by their characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Case group N = 56</th>
<th>Control group N = 27</th>
<th>Chi square test</th>
<th>Ratio (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive 1-hydroxy pyrene in urine</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>69.6</td>
<td>16</td>
<td>59.3</td>
</tr>
<tr>
<td>Females</td>
<td>17</td>
<td>30.4</td>
<td>11</td>
<td>40.7</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>21</td>
<td>37.5</td>
<td>9</td>
<td>33.3</td>
</tr>
<tr>
<td>Urban</td>
<td>35</td>
<td>62.5</td>
<td>18</td>
<td>66.7</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>28</td>
<td>50.0</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>Non smoker</td>
<td>28</td>
<td>50.0</td>
<td>17</td>
<td>63</td>
</tr>
<tr>
<td>Type of hepatitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>21</td>
<td>37.5</td>
<td>7</td>
<td>26.0</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>35</td>
<td>62.5</td>
<td>20</td>
<td>74.0</td>
</tr>
<tr>
<td>Alpha Feto Protein (AFP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1</td>
<td>1.8</td>
<td>23</td>
<td>85.2</td>
</tr>
<tr>
<td>Elevated</td>
<td>55</td>
<td>98.2</td>
<td>4</td>
<td>14.8</td>
</tr>
<tr>
<td>Signs of decompensation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>35</td>
<td>62.5</td>
<td>25</td>
<td>92.6</td>
</tr>
<tr>
<td>Absent</td>
<td>21</td>
<td>37.5</td>
<td>2</td>
<td>7.4</td>
</tr>
</tbody>
</table>

*Significant

DISCUSSION

Regarding biological monitoring of 1-Hydroxy pyrene in urine as a biomarker of exposure to Polycyclic Aromatic Hydrocarbons (PAHs) among the studied groups (Table 1), there was significant association between exposure to PAHs and development of HCC (OR = 4.9). This agreed with Lee et al. (2009), Shahataheri (2009), Van Larebeke et al. (2010), and Hansen et al. (2013). They reported the exposure to PAHs might be a risk factor of lung, kidney, renal and testicular cancer. Also, agreed with Jing Yang et al. (2017) who concluded that the data of their study reinforce that urinary 1-hydroxy pyrene can be a useful biomarker for evaluating total PAHs exposure and in assessing the effect of PAHs exposure on oxidative damage. This agreed with Guohang et al. (2012) who concluded that PAHs are found in the human rectal tissues or hepatic tissues. The content of PAHs in the human rectal tissues may have affection on the occurrence of rectal cancer while the content of PAHs in the hepatic tissues may have ones.
Concerning the relation of 1-Hydroxy pyrene in urine and the characteristics of the studied groups, there was significant statistical association between the presence of 1-Hydroxy pyrene in urine and sex of examined subjects and males had 1.65 fold more than females to develop HCC on top of chronic active hepatitis B and C when exposed to PAHs (OR = 1.6). This agreed with Dong and Lee (2009) who reported that positive association between male and exposure to PAHs for development of cancer. But this disagreed with Oanh et al. (2009) who found no association between sex and exposure to PAHs for development of cancer. The discrepancies in the result of that study and other ones might be attributed to the type of study population. In the present study, the target population was those of hepatitis with the cases were those with HCC. Also, the rate may varies in the discrepant studies. In some studies, it was found that higher concentrations of exposure to PAHs were among females than compared with males, likely due to more exposure from the cooking stoves and wood combusted heating in certain communities (Zhang et al., 2014).

In the view of the relation of 1-Hydroxy pyrene in urine and the residence of the examined groups (Table 2), it was found no statistically significant difference between the two groups, and no association between exposure to PAHs in urban areas and development of HCC on top of chronic active hepatitis B and C in the case group (OR =0.8). This disagreed with the results of Dong and Lee (2009) who stated that increase the risk of cancer due to exposure to PAHs in the urban areas and explained that by more exposure to industrial and mobile sources.

As regards the relation of 1-Hydroxy pyrene in urine and smoking habit among the examined groups. Although no statistically significant difference between the two groups, there was positive association between smoking and development of HCC on top of chronic active hepatitis B and C in the case group when exposed to PAHs (OR = 1.7). This agreed with Yang et al. (2009), Li & Ro (2010), and Poppi and Silva (2015) who reported that positive association between smoking and development of HCC among cases of chronic active hepatitis B or C. This agrees also with Jing Yang et al., 2017) who found that smoking can significantly increase the level of 1-hydroxy pyrene in urine in cases exposed to PAHs and smoking will cause more serious DNA oxidative damage among the exposed cases to PAHs and agreed with Zhu et al. (2011) who concluded that the major route of exposure to PAHs in the general population is from breathing ambient air polluted with PAHs and indoor eating food containing PAHs, cigarettes smoking or breathing smoke from open fire.

Regarding the relation of 1-Hydroxy pyrene in urine and different types of hepatitis among the examined groups, there was neither statistical significance difference between the two groups nor association between cases of chronic active hepatitis C and development of HCC when exposed to PAHs. This might be explained by effect of hydrocarbons in development of hepatic cancer not determined with type of viral hepatitis.

As regards the relation of 1-Hydroxy pyrene in urine and AFP among the examined groups, there was a highly
positive association between abnormal level of 1-Hydroxy pyrene in urine and elevated AFP and there was a statistical significance difference between exposed and non-exposed groups. This could be attributed to exposure to PAHs has a positive effect on the level of AFP. Chronic exposure AFP is a major risk factor for HCC; increased risk has been linked to polycyclic aromatic hydrocarbon (PAH) co-exposure and hepatitis virus infection (Johnson et al., 2010). Furthermore, since hepatitis viruses are major etiological factors for liver cancer (McGlynn et al., 2015 and Makarova-Rusher et al., 2016) and HBV infection interacts with AFP exposure to greatly increase liver cancer risk (Smith et al., 2017), hepatitis virus exposures should be assessed in conjunction with AFP.

Concerning the relation of 1-Hydroxy pyrene in urine and signs of decompensation among the examined groups, there was negative association between the two abnormalities. Although no similar studies in the literature have found to address this point, the explanation of this finding might be attributed to liver state of cirrhosis and cancer not the exposure to PAHs. PAH metabolism usually occurring in the liver like most xenobiotics and hydrocarbons. The enzyme system that is primarily responsible for PAH metabolism is the mixed-function oxidase system (Hodgson and Rose, 2010 and Wohak et al., 2016). The progress of liver fibrosis and cirrhosis is accompanied by deteriorating liver function, including functional changes in phase I and phase II metabolic enzymes (Dietrich et al. 2016).

CONCLUSION

Cases of HCC had increased level of 1-hydroxy pyrene in urine with statistical significance difference when compared to controls. There was a significant positive association between exposure to PAHs and development of HCC among males and smokers. There was neither statistical significance difference nor positive association between exposure to PAHs and development of HCC in urban areas and among patients with chronic active hepatitis. There was a highly positive correlation between 1-hydroxy pyrene and Alfa Feto Protein (AFP) among positive cases of 1-hydroxy pyrene in case group.

RECOMMENDATIONS

Elimination of exposure to PAHs is needed. Environmental monitoring of PAHs in different residential areas in different governorates for detection of source of pollution with PAHs in air, soil and water is needed.

REFERENCES


المراجعة البيولوجية للهيدروكوربونات العطرية متعددة الحلقات كعامل
خطر محتمل لسرطان الخلايا الكبدية بين حالات التهاب الكبد المزمن النشط ب وس

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خلفية البحث: تعتبر الهيدروكوربونات العطرية متعددة الحلقات من بين أكثر
الملوثات المسؤولة للسرطان والมะفزة والسماء، و التعرض والتمثيل الغذائي لهذه
المواد يعطى نتائج الضعى النووي التفاعلي في الجسم مسببًا للعديد من أنواع
السرطان البشري.

هدف البحث: تهدف هذه الدراسة إلى معرفة ما إذا كان التعرض
للهيدروكوربونات العطرية متعددة الحلقات عاملًا خطأً لنمو سرطان الخلايا
الكبدية في الحالات المعرضة. و اكتشاف ما إذا كان التدخين هو عامل خطورة
لزيادة حدوث سرطان الكبد في الحالات المعرضة. و لمعرفة تأثير الخصائص
الديموغرافية الاجتماعية لحالات سرطان الكبد المعرضين للهيدروكوربونات
العطرية متعددة.

الأشخاص وطريقة البحث: أجريت دراسة لمراجعة الحالات بين الفترة من الأول
من مارس 2015 وحتى نهاية أغسطس 2017. وقد أجريت الدراسة في العيادات
الخارجية التابعة لقسم أمراض الكبد والجهاز الهضمي في معهد تيودور بلهارس
للإبحار. و قد تم حساب الحد الأدنى لحجم العينة المطلوبة لهذه الدراسة باستخدام
برنامج الأبي انفو، مع مراجعة البيانات التالية: مستوى الثقة من الجنبين = 95%.
قوة الاختيارات = 80 %، نسبة الحالات: الضوابط = 1:1، نسبة الضوابط
المعرضين = 21 %، نسبة الحالات المعرضين = 42 %، و قد تم تقدير عدد
النتائج: أوضحت النتائج بأن 73% من حالات سرطان الكبد لديهم زيادة في مستوى مادة 1-هيدروكسي بيرين في البول مع اختلاف ذو دلالات إحصائية عند مقارنتها بالضحايا. وكان هناك ارتباطًا كبيرًا بين التدخين وزيادة مستوى مادة 1-هيدروكسي بيرين في البول. وكان هناك ارتباطًا إيجابيًا كبيرًا بين التعرض للهيدروكربونات العضوية متعددة الحلقات ونمو سرطان الكبد في الذكور (نسبة أرجحية = 1.6). بينما لم يكن هناك فرق ذو دلالات إحصائية، ولا علاقة إيجابية بين التعرض للهيدروكربونات العضوية متعددة الحلقات وسرطان الكبد بالنسبة للسكان في المناطق الحضرية. وقد كان هناك ارتباطًا إيجابيًا بين التعرض للهيدروكربونات العضوية متعددة الحلقات وحدوث سرطان الخلايا الكبدية بين المدخنين (نسبة أرجحية = 1.7). كما كان هناك ارتباطًا إيجابيًا قويًا بين التعرض للهيدروكربونات العضوية متعددة الحلقات ونسبة بروتين ألفافيتو بالدم (نسبة أرجحية = 316.25).

الاستنتاج: يعد التعرض للهيدروكربونات العضوية متعددة الحلقات عامل خطورة لحدوث سرطان الخلايا الكبدية بين حالات إثبات الكبد المزمن النشط ب وسي. وقد زادت نسبة مستوى 1-هيدروكسي بيرين في البول في حالات سرطان الكبد مع وجود اختلاف ذو دلالات إحصائية عند مقارنتها بالضحايا. كما تم الكشف عن وجود ارتباط إيجابي بين التعرض للهيدروكربونات العضوية متعددة الحلقات وسرطان الكبد في الذكور والمدخنين.