Sys Rev Pharm 2021;12(1):726-732
A multifaceted review journal in the field of pharmacy

The Levels of Acrylamide and Glycidamide as Biomarker in Smokers: An Article Review

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ABSTRACT

Cigarette smoke is the major source of acrylamide exposure after food. Acrylamide is classified as a probable carcinogenic to humans. Acrylamide is metabolized by CYP2E1 into glycidamide that is very reactive to DNA and can form DNA adducts causing carcinogenic effects in humans. Acrylamide levels in cigarette smoke is around 1.000–7.991 µg/cigarette. Acrylamide exposure in smokers is 2.2–4.6 times higher than non-smokers and glycidamide exposure 1.1–3.8 times higher than non-smokers. Acrylamide and glycidamide are found in blood, body tissues, placenta, and breast milk. The levels of acrylamide and glycidamide in Dried Blood Spot (DBS) sample are still unknown. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) can be used to quantify small amounts of analytes. Volumetric application or microfluidic DBS used to overcome hematocrit effect.

Keywords: Acrylamide, Glycidamide, Smoker, Dried Blood Spot, LC-MS/MS

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INTRODUCTION

Tobacco is one of the biggest causes of health problems in the world. Tobacco kills more than 8 million people every year. More than 7 million deaths are caused by direct tobacco use and around 1.2 million deaths are caused by exposure to other people's cigarette smoke (WHO, 2019) [1]. More than 7000 chemicals found in cigarette smoke and 69 of them can cause cancer. Acrylamide is one of the carcinogenic chemicals in cigarette smoke that has not been much studied compared to other carcinogenic chemicals in cigarette smoke[2].

Acrylamide is classified as a probably carcinogenic to humans (Group 2A) [3]. Acrylamide is metabolized by CYP2E1 and forms glycidamide, an epoxide that very reactive to DNA. Glycidamide can bind to DNA to form N7-(2-carbamoyl-2-hydroxyethyl) guanine (N7-GA-Gua) and N3-(2-carbamoyl-2-hydroxyethyl) adenine (N3-GA-Ade). This can be a major cause of mutagenicity and carcinogenesis due to acrylamide exposure [4],[5]. DBS as the biosampling method can be used to determine the levels of acrylamide and glycidamide. The blood is collected by finger prick and only few microliters of blood are dropped onto DBS paper [6]. LC-MS/MS is a sensitive and selective method for quantification of analytes in DBS samples. LC-MS/MS can separate compounds very well in a short time [7].

Moldoveanu *et al* found that acrylamide levels in cigarette smoke are more than 1 µg[8]. Analysis of biomarkers in smokers and non-smokers has been done by Harahap *et al* and found acrylamide levels in lung cancer patients with smoking record are 7.64 µg/mL and negative blanks are 2.72 – 3.51 µg/mL[9]. Zhang *et al* found hemoglobin adduct of acrylamide (HbAA adduct) and glycidamide (HbGA adduct) levels in smokers as much as108.7 ± 29.5 pmol/g Hb and 58.3 ± 25.1 pmol/g Hb while in non-smokers as much as 24.2 ± 12.5 pmol/g Hb and 52,0 ± 33,6 pmol/g Hb[10]. Vesper *et al* found HbAA and HbGA adduct levels in smokers as much as 194 pmol/g Hb and 107 pmol/g Hb while non-smokers are 51 pmol/g Hb and 34 pmol/g Hb[11]. Boettcher *et al* found mercapturic acid *N*-acetyl-S-(2-carbamoylethyl)-l-cysteine (AAMA) and *N*-acetyl-*S*-(2-carbamoyl- 2-hydroxyethyl)-l-cysteine (GAMA)levels in smokersas much as127 µg/L and 19 µg/L whilein non-smokers are 29 µg/L and 5 µg/L[12]. Huang *et al* also found N7-GA-Gua levels in smokers as much as2.01 µg/L urine and non-smokers is 1.5 µg/L urine[13]. In 2020, Harahap *et al* analyze acrylamide levels of lung cancer patients with smoking record in DBS with LC-MS/MS. The LLOQ of acrylamide is 2.5 µg/mL.The results showed that acrylamide levels in DBS samples of lung cancer patients with smoking record were higher than those without smoking record [9]. Glycidamide levels is smaller than parent compound, acrylamide, because acrylamide is converted to glycidamide at least 6%. Therefore, LC-MS/MS is needed to detect small compounds in the blood sample[14].

Zhivagui *et al* revealed the association between acrylamide and cancer in humans. She found the glycidamide mutational signature in one-third of 1600 tumor genomes that matched to 19 types of tumors from 14 human organs[15]. Based on the data above, it is very important to study the acrylamide and glycidamide. This review article is expected to attract public attention especially smokers to reduce or avoid the consumption of cigarettes to prevent various diseases such as cancer.

Chemicals in cigarette smoke

Cigarette smoke contains many carcinogenic substances including 10 species of polynuclear aromatic hydrocarbons (PAHs), six species of heterocyclic hydrocarbons, four species of volatile hydrocarbons,three species of nitrohydrocarbons, four species of aromatic amines, eight species of N-heterocyclic amines, 10 species of N-nitrosamines,two species of aldehydes, 10 species of miscellaneous organic compounds, nine species of inorganic compounds and three phenolic compounds[3]. Table A.1 shows all the carcinogenic compounds in cigarette smoke. Cigarette smoke can cause cancer in human body, including lung, oral cavity, nasopharynx, oropharynx, hypopharynx, nasal cavity, sinus, larynx, esophagus, stomach, pancreas, colorectum, liver, kidney, ureter, bladder, cervix, ovarian, and myeloid leukemia[16].

Acrylamide

Acrylamide, an organic compound known as 2-propenamide, has the molecular formula C_3H_5NO. Acrylamide is a solid monomer, white, and odorless crystalline [17]. Acrylamide is classified as probably carcinogenic to humans (Group 2A)[3]. Acrylamide is a conjugated vinyl compound that is reactive to biological materials. Acrylamide has amine group and vinyl group, so it is an electrophilic compound. The double bond has a strong interest toward –SH groups in cysteine and $–NH_2$ groups in proteins or amino acidsvia Michael-type nucleophilic addition reactions [18]. Fig. A.1 shows chemical structure of acrylamide[19].

Source of Acrylamide

The source of acrylamide is from exposure to cigarette smoke, food, and environment. The main source of acrylamide is foodwhich is daily intake of people[20].According to the FAO/WHO Joint Experts Committee for Food Additives, the average intake of acrylamide through food is around 0.2 – 1 µg/kgBW/day[14]. The average intake of acrylamide in Sweden is 0.5 µg/kgBW/day while in Germany and Europe is 0.3 – 0.8 µg/kgBW/day [20, 21]. Boettcher *et al* revealed the average exposure to acrylamide from cigarette smoke was three times higher than food. Exposure to acrylamide from cigarette smoke is 3 µg/kgBW/day while from food is 1 µg/kgBW/day[12]. It can be the main reason that cigarette smoke is the main source of acrylamide exposure after foods.

Acrylamide levels in cigarette smoke

Acrylamide levels in cigarette smoke is 1.10 – 2.34 µg/cigarette [13, 17, 22]. The other sources say that acrylamide levels in cigarette is 1 – 2 µg/cigarette [11, 12]. Acrylamide levels in commercial cigarette smoke from the US market, 2R4F, and 3R4F is more than 1 µg/cigarette [8]. In 2014, Papoušek *et al* determine acrylamide levels in cigarette smoke using smoking simulation equipment. Acrylamide levels in mainstream smoke is 2.40 µg/cigarette and side stream smoke is 1.52 µg/cigarette [21].

Variation of acrylamide levels in cigarette smoke can be caused by differences cigarette brands from various countries. Acrylamide levels in cigarette smoke from US is 1.000 – 2.700 µg/cigarette and from China is 4.753 – 7.991 µg/cigarette[20]. Based on the data above, it can be concluded that acrylamide levels in cigarette smoke is around 1.000– 7.991 µg/cigarette.

Biotransformation of acrylamide to glycidamide

Biotransformation of acrylamide to glycidamide can be seen in the Fig. A.2[23]. Metabolism of acrylamide occurs via epoxidation to glycidamide or conjugation with glutation (GSH). Acrylamide is metabolized by CYP2E1 to glycidamide[22]. Glycidamide has an epoxide ringthat very reactive when it is open. Fig. A.3 shows chemical structure of glycidamide[24]. Reactivity of glycidamide to DNA is higher than acrylamide and glycidamide can form DNA *adduct,* N7-GA-Guaand N3-GA-Ade[25]. Zhivagui *et al* revealed the association between acrylamide and cancer in humans. She found that the glycidamide mutational signature in one-third of 1600 tumor genomes matched to 19 types of tumors from 14 human organs. The highest enrichment of the glycidamide signature was observed in the cancers of the lung (88%), liver (73%), kidney (>70%), bile duct (57%), and cervix (50%)[15].

Detoxification of acrylamide and glycidamide by conjugating with GSH catalyzed by GST forms mercapturic acid metabolites (AAMA, GAMA, and iso-GAMA). Glycidamide can also be hydrolyzed by epoxide hydrolase to form glyceramide. AAMA, GAMA, iso-GAMA, and glyceramide can be excreted through urine[26].

The levels of acrylamide and glycidamide levels in smokers and non-smokers

Acrylamide and glycidamide are the important compounds to be used as biomarkers to assess potential risk of acrylamide exposure. Many studies have measured the levels of acrylamide and glycidamide in smokers as positive subjects and non-smokers as negative subjects and then compared the two groups of subjects. Analysis can provide more accurate results by applying subject selection criteria. These criteria include smoking habits (brand and number of cigarettes consumed and since when consuming cigarettes) and eliminating other sources of acrylamide exposure, such as food and environment.

Harahap *et al* found acrylamide levels in lung cancer patients with smoking record is 2.2 – 2.8 which is higher than negative blanks. Acrylamide levels in lung cancer patients is 7.64 µg/mL and negative blanks is 2.72 – 3.51 µg/mL[9]. Schettgen *et al* conducted a study with 29 subjects tested and 16 of them smokers (consumption of 10 cigarettes/day). They found HbAA and HbGA adduct levels in smokers are 80 and 53 pmol/g while non-smokers are 19 and 17 pmol/g globin[27]. Vesper *et al* found HbAA and HbGA adduct levels in smokers are 194pmol/g and 107pmol/g while non-smokers are 51pmol/g and 34pmol/g globin[11]. Zhang *et al* found HbAA and HbGA adduct levels in smokers are 108.7 ± 29.5 and 58.3 ± 25.1 pmol/g Hb while non-smokers are 24.2 ± 12.5 pmol/gHb and 52.0 ± 33.6 pmol/g Hb[10]. Determination of acrylamide exposure through Hb adduct shows that acrylamide exposure in smokers is 2.2 – 4.6 times higher than non-smokers and glycidamide exposure is 1.1 – 3.8 times higher than non-smokers.

Boettcher *et al* conducted a study with 29 subjects tested and 13 of them are smokers (consumption of 10 cigarettes/day). They found AAMA and GAMA levels in smokers are 127µg/L and 19µg/L while non-smokers are 29µg/L and 5µg/L[12]. Urban *et al* also conducted a study with 120 subjects tested and 60 of them smokers. They found AAMA and GAMA levels in smokers are 107.3µg/L and 15µg/L while non-smokers are 41.6µg/L and 8.7µg/L[28]. Bjellaas *et al* found mercapturic acidlevels in smokers is four times higher than non-smokers. AAMA level in smokers is 74 µg/L while in non-smokers is 16 µg/L[29]. Determination of acrylamide exposure through mercapturic acid metabolites shows that acrylamide exposurein smokers is2.6– 4.6 times higher than non-smokers and glycidamide exposure is 1.7– 3.8 times higher than non-smokers.

Huang *et al* (2015) have analyzed N7-GA-Gua in urine samples of 30 smokers and 33 non-smokers. N7-GA-Gua level in smokers is 2.01 µg/L while non-smokers is 1.5 µg/L. However,assuming that50% of acrylamide intake is converted to AAMA, the formation of urinary N7-GA-Gua is estimated at around 0.66% of the daily intake of acrylamide[13]. Determination of acrylamide exposure through N7-GA-Gua showed that glycidamide exposure in smokers is 1.3 times higher than non-smokers.

Variation of acrylamide and glycidamide levels in human body

Variations of acrylamide and glycidamide levels may result from genetic activity and/ or polymorphismin metabolism, detoxification, and DNA repair[13]. Enzyme activity is affected by genetic factors and habits such as

alcohol and smoking [11, 21]. People with rare syndromes like *Xerodermapigmentosum* have deficient DNA repairso they are highly prone to cancer development. When DNA adduct persist unrepaired, then they can cause miscoding during DNA replication and resulting permanent mutation [30]. Pelle *et al* found that T-allele of rs2480258 can decrease CYP2E1 activity[26].

Dried Blood Spot (DBS)

DBS is a biosampling method where blood samples are collected by finger prick. DBS has many advantages over conventional methods, including convenient for subjects, small sample volumes, analytes are usually more stablebecause they are dried, simple sample preparation, easy storage and transport while whole blood samples must be kept at 2 – 10^{o}C [6, 31]. The challenge of using DBS is small sample size that contain the small analytes. It can be overcome by combining DBS with LC-MS/MS. LC-MS/MS can quantify small analytes in DBS with a sensitive and selective method[31].

The other challenge of using DBS is homogeneity of blood spots.Variations of distribution and non-homogeneity of the blood can be overcome by taking all the blood spots (not only based on the size of the punch or a certain diameter) to give consistent concentration of analytes regardless of the hematocrit effect[32]. There are several ways to overcome hematocrit effect such as volumetric application. Harahap *et al* used volumetric application where blood was collected in vacutainer tube and pipetted with volumetric pipette to DBS paper. Modern devices can also be used to overcome hematocrit effect such as Hema PEN, Volumetric Absorptive Microsampling (VAMS), Volumetric Absorptive Paper Disc (VAPD), mini-disc (VAPD mini), and microfluidic DBS (example: Hema Xis DB and Capitainer-B)[33]. Over time, modern microfluidic DBS has become an alternative for collecting blood sample. Spooner *et al* and Lenk *et al* proved that the use of microfluidic DBS gives great results as good as the use of volumetric pipettes [34, 35]. Spooner *et al* found that the use of microfluidic DBS gives constant blood volume with low variability, same accuracy, and better precision than volumetric pipettes[34].

Both conventional methods using volumetric pipettes and modern methods of microfluidic DBS are effective in removing the hematocrit effect. Microfluidic DBS is very easy to use by the subjects themselves. However, higher costs are certainly unavoidable. The use of volumetric pipettes minimizes costs and blood collection is very easy if someone is professional enough like storing blood in a vacutainer tube containing anticoagulants, piping techniques, and proper bottling techniques on DBS paper.

Method of acrylamide and glycidamide analysis

Various methods have been developed to analyze acrylamide and glycidamide simultaneously in various matrices, both in animals and humans using LC-MS/MS as conducted by Kim *et al*, Zhang *et al*, Boettcher *et al*, and Huang *et al* [5, 10, 12, 13]. Many studies using acrylamide and glycidamide as biomarkers show that glycidamide metabolites are also important as biomarkers for acrylamide exposure because glycidamide has the risk of forming DNA adducts and causing cancer. However, no one has developed a method for simultaneous analysis of acrylamide and glycidamide in DBS. Nevertheless, there is already an acrylamide analysis method in DBS that is used by Harahap *et al* [9].

LC-MS/MS

LC-MS/MS is a separation method that can be used for acrylamide. LC-MS/MS is better than GC-MS because it does not need derivatization process and facilitates the separation of compounds due to good separation, Short retention time, high sensitivity and high selectivity [6, 36]. Other studies on the analysis of acrylamide and glycidamide using LC-MS/MS have also been carried out by Zhang *et al*. They analyses the levels of HbAA and HbGA adducts in smoker's blood[10]. Boettcher *et al*, Bjellaas *et al,* and Huang *et al* analyzes of mercapturic acid metabolitesin smoker's urine [11, 12, 29]. Huang *et al* analyzes N7-GA-Gua levels in smoker's urine[13].

Measurement the levels of acrylamide and glycidamide

Method validation can be developed to analyze acrylamide and glycidamide simultaneously in DBS using LC-MS/MS. The parameters must in accordance with the FDA 2018 guidelines for full validation in term of selectivity, carryover, sensitivity, linearity, accuracy, precision, recovery, matrix effects, dilution integrity, and stability[37]. Validated methods can be used to analyze acrylamide and glycidamide in smokers and non-smokers.

CONCLUSION

Acrylamide and glycidamide are the important biomarkers for assessing potential risk of acrylamide exposure. Acrylamide levels in cigarette smoke is around 1.000 – 7.991 µg/cigarette caused by different cigarette brands. Acrylamide exposure in smokers is 2.2 – 4.6 times higher than non-smokers and glycidamide exposure is 1.1 – 3.8 times higher than non-smokers. DBS has many advantages over conventional methods, including convenient for subjects, small sample volumes, analytes are more stable, easy storage and transport, and simple sample preparation. Volumetric application by using volumetric pipettes or modern devices such as microfluidic DBS are used to overcome hematocrit effect. LC-MS/MS method can quantify small amounts of analytes due to good separation, short retention time, high sensitivity, and high selectivity.

Author's contributions

All authors contributed equally to this review.

COMPETING INTEREST

There is no conflict or competing interest among the authors.

Table A.1.Carcinogens in cigarette smoke

Agent	IARC Group
Polynuclear aromatic hydrocarbons (PAH)	
Benz[*a*]anthracene	2A
Benzo[b]fluoranthene	2B
Benzo[j]fluoranthene	2B
Benzo[k]fluoranthene	2B
Benzo[a]pyrene	2A

Dibenz[a,h]anthracene	2A
Dibenzo[a,i]pyrene	2B
Dibenzo[a,e]pyrene	2B
Indeno[1,2,3-cd] pyrene	2B
5-Methylchrysene	2B
Heterocyclic hydrocarbons	
Furan	2B
Dibenz(a,h)acridine	2B
Dibenz(a,j)acridine	2B
Dibenzo(c,g)carbazole	2B
Benzo(b)furan	2B
N-Nitrosamines	
N-Nitrosodimethylamine	2A
N-Nitrosoethylmethylamine	2B
N-Nitrosodiethylamine	2A
N-Nitrosopyrrolidine	2B
N-Nitrosopiperidine	2B
N-Nitrosodiethanolamine	2B
N'-Nitrosonornicotine	2B
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	2B
Aromatic amines	
2-Toluidine	2A
2,6-Dimethylaniline	2B
2-Naphthylamine	1
4-Aminobiphenyl	1
***N*-Heterocyclic amines**	
A-α-C	2B
MeA-α-C	2B
IQ	2A
Trp-P-1	2B
Trp-P-2	2B
Glu-P-1	2B
Glu-P-2	2B
PhIP	2B
Aldehydes	
Formaldehyde	2A
Acetaldehyde	2B
Phenolic compounds	2B
Catechol	2B
Caffeic acid	
Volatile hydrocarbons	
1,3-Butadiene	2A
Isoprene	2B
Benzene	1
Nitrohydrocarbons	
Nitromethane	2B
2-Nitropropane	2B
Nitrobenzene	2B
Miscellaneous organic compounds	
Acetamide	2B
Acrylamide	2A
Acrylonitrile	2B
Vinyl Chloride	1
1,1-Dimethylhydrazine	2B
Ethylene oxide	1
Propylene oxide	2B
Hydrazine	2B
Urethane	2B

Metals and metal compounds	
Arsenic	1
Beryllium	1
Nickel	1
Chromium	1
Cadmiun	1
Cobalt	2B
Lead (inorganic)	2A
Radioisotope	
Polonium-210	1

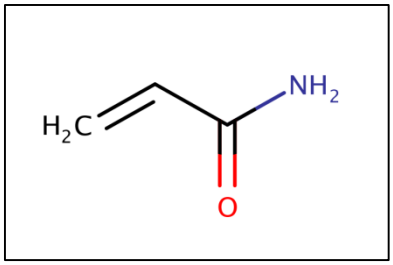

Fig. A.1.Chemical Structure of Acrylamide[19]

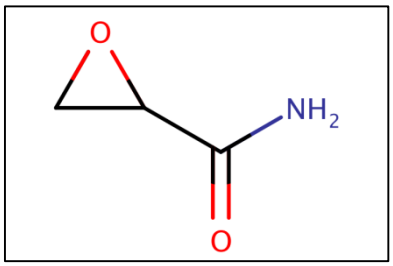

Fig. A.2. Chemical Structure of Glycidamide[24]

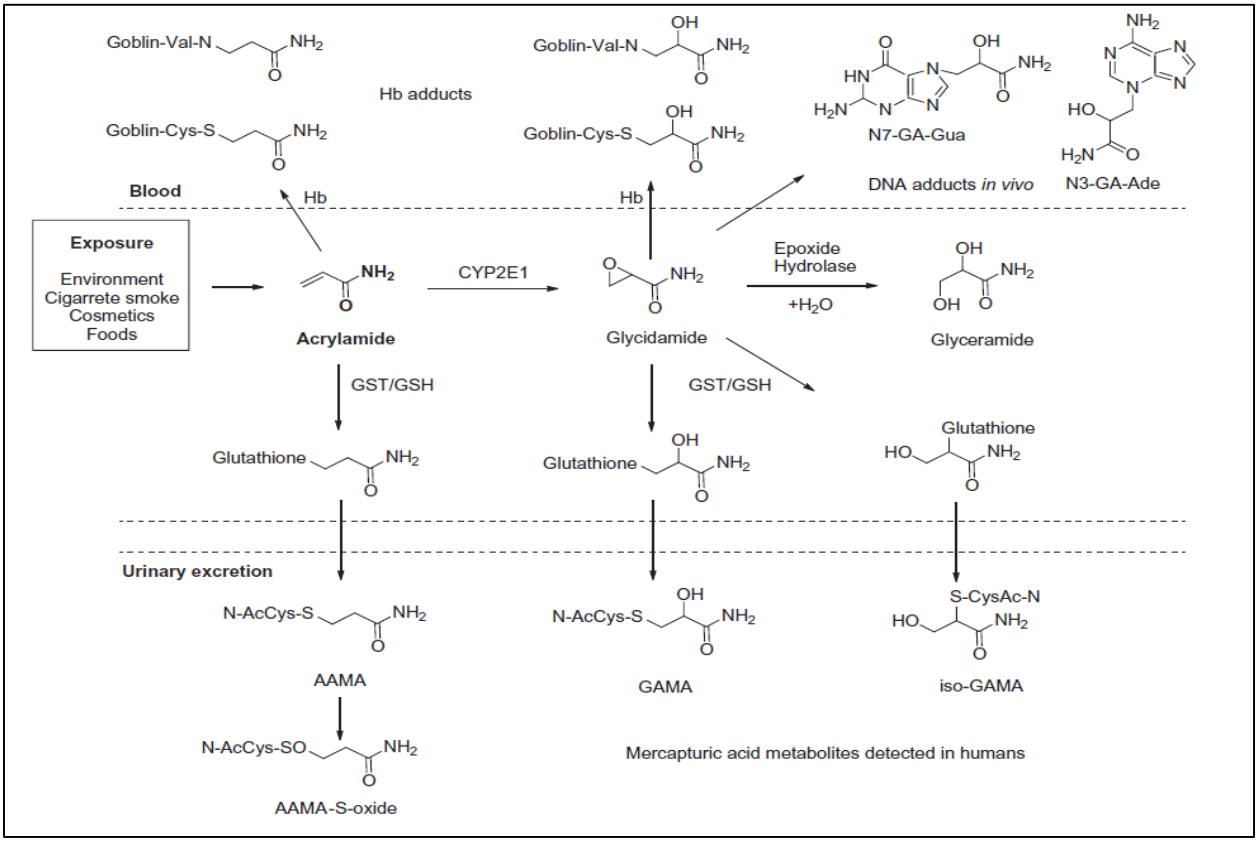

Fig. A.3. Biotransformation of Acrylamide and Glycidamide[23]

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