Female drinking, environment and biological markers

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Summary. The rate of women involved in alcohol abuse is rapidly increasing and the age of first use tends dramatically to decrease. The health and social costs are high both for the adverse effects on physical and psychological woman health, and for the teratogenic effect of alcohol on fetal development. The review takes in account physiological aspects of alcohol effects according to age and gender differences. Interaction between alcohol habit and environment are discussed together with the risk of co-exposure to alcohol and pollutants. The role of biomarkers may be invaluable for clinical utility, prevention and early intervention above all to avoid prenatal, not reversible damages. The update of alcohol studies shows the greater severity of alcohol damage in female and the need of gender-targeted intervention.

Key words: alcohol, woman, gender, biomarker, environment, prenatal exposure.

Riassunto *(Bere femminile, ambiente e indicatori biologici)*. L'aumento delle donne coinvolte in problemi alcol relati e il drammatico abbassamento dell'età di primo uso, costituiscono un problema di sanità pubblica sia per gli effetti sulla salute fisica e psichica della donna, sia per gli effetti teratogeni dell'alcol assunto in gravidanza sullo sviluppo del feto. Il lavoro presenta gli effetti fisiologici dell'alcol in considerazione delle differenze di genere e di età, e i fattori di rischio per la salute dovuti all'interazione tra assunzione alcolica e contaminanti ambientali. Il ruolo degli indicatori biologici può essere essenziale nella pratica clinica, nella prevenzione e nell'intervento precoce soprattutto per evitare danni prenatali non reversibili. Gli studi sull'alcol concordano sulla maggiore severità del danno alcolico nella donna e sulla necessità di interventi specifici per il genere.

Parole chiave: alcol, donna, indicatori biologici, ambiente, esposizione prenatale.

INTRODUCTION

Excessive drinking is a major health problem worldwide and could be considered a social "emergency". Alcohol problems rise both from alcoholic beverages diffusion and from changes in drinking habits that are involving young people and women more and more. Alcohol related problems are generated by a lot of factors with personal, social, health aspects. The approach to alcohol problems tended to be socio-ethical rather than scientific since the availability of alcoholic beverages, social acceptance, traditional use into the family makes the perception of risk of alcohol misuse very difficult. The concept itself of excessive drinking is debated and even some health operators have difficulties in the correct evaluation and approach to alcohol problems [1].

Ethanol exposure, together with other environmental risky factors due to lifestyle (tobacco, drugs use) and to exposure to pesticides, heavy metals, pollutants etc., has an amplifying action on health damage in addiction to ethanol "reinforcing" properties on brain functions. Information about side effects due to simultaneous exposure to ethanol and environmental toxics are not well known, but a synergic action is likely.

The effects on public health may be heavy especially for women and children. Several studies support the hypothesis that women are more vulnerable to ethanol effects for their physiological, metabolic, hormonal peculiarities [2, 3], and the increase of female drinking is an emerging alert in our days. USA epidemiological studies in the early '80s revealed a situation of alcohol abuse and dependence for the 23% of men and 4% of women (ratio = 5.75); ten years after the same problems involved the 20% of men and 8% of women (ratio = 2.50). At the end of 90's the ratio men/women among at-risk drinking adolescents (teen agers 12-17 yrs), was close to 1:1 [4, 5].

Epidemiological studies across Europe confirm that, in 2004, the rate of at risk drinking female was increasing more quickly than men and that the rate among female teenagers was quite similar (or outstripping) to male [6].

Children may be exposed to alcohol effects from conception, as maternal alcohol drinking during pregnancy may have dramatic effects [7]. They cannot be considered "little adults" in environmental medicine, since there are differences in exposure, pathways of

absorbtion, tissue distribution, ability to biotransform or eliminate chemicals, responses to chemical and radiation. The differences vary with the developmental stage of child and little is known about the impact of environmental factors on children [8]. The epidemiological data, pointing out the increase of female drinking, support the alert for the health of woman and child.

Self-report questionnaires are used to evaluate drinking habits and the type of alcoholic beverage taken, but their reliability is questionable, since most alcoholics are quite reticent about their drinking patterns and their real alcohol consumption. The role of excess drinking biomarkers, together with clinical examination, instrumental data and psychological tests as MAST (Michigan Alcoholism Screening Test), MALT (Munich Alcoholism Test), SADQ (Severity of Alcohol Dependence Questionnaire), AUDIT (Alcohol Use Disorders Identification Test) may be essential to ascertain and evaluate at risk drinking patterns and to identify health hazard for women, pregnant women and children [9].

Early identification of risky behavior could prevent prenatal damage and could avoid or minimize disabilities, mental health problems or trouble with the law in the future adults. Furthermore advances in the field of biomarkers of exposure, effect and susceptibility, may have important implications for the detection, prevention and treatment of environmentally induced disabilities.

This paper focuses on some aspects of alcohol-related problem in woman during adolescence, reproductive years and older age. Peculiarities of female drinking and possible interactions between alcohol and environmental toxics will be considered here, as well as the role of biomarkers in evaluating alcohol-related health hazard.

FEMALE DRINKING AND METABOLISM

Blood alcohol concentration (BAC) depends on alcohol ingested and adsorbed by gastrointestinal tract, by volume of distribution in the body and by elimination rate. The higher will be BAC, the greater will be ethanol effects. Ethanol is adsorbed in different way depending on concentration and amount of alcohol ingested, on amount and type of food (if any) in the stomach, on drinking pattern.

Most tissues of the body contain enzymes capable of ethanol oxidation or non-oxidative metabolism, but significant activity occurs only in the liver and, to a lesser extent, in the stomach. Hence, medical consequences are predominant in these organs. In the liver, ethanol oxidation generates an excess of reducing equivalents, mainly NADH, causing hepato-toxicity. An additional system, containing cytochromes P-4502E1 (CYP2E1) inducible by chronic alcohol feeding, was demonstrated in liver microsomes and is a major cause of hepatotoxicity [10]. CYP2E1 is also induced in Kupffer cells, promoting their activation and the release of inflammatory cytokines, including tumor necrosis factor (TNF)-α [11].

Before metabolism by the liver, during the first pass metabolism (FPM) in the stomach ethanol is metabolized by the gastric isoenzyme alcohol dehydrogenase (ADH). Finally, ethanol is metabolized by hepatic ADH.

The enhanced vulnerability of women to alcohol-related damage may be due to their higher blood alcohol levels after drinking, but the mechanisms are debated. BAC is strongly dependent on body mass index (BMI) and body water. Female BMI and total body water are lower than male, leading to lowered ethanol diffusion in the body and resulting in higher BAC. If BAC value was normalized according to content of total body water, gender differences were flattened [12,13]. Several years ago it was demonstrated that activity of gastric ADH responsible for FPM is significantly lower in females than in males and is close to zero in heavy drinking females [14].

Thus, in woman a larger amount of alcohol ingested will reach the liver directly, promoting a more rapid progression of liver damage. A recent experimental study demonstrated that the gender differences in alcohol levels is due mainly to a significantly lesser activity of female χ-ADH, rather than to differences in gastric emptying or in hepatic oxidation of ethanol. These effects were demonstrated to be concentration-dependent, since women had a gastric first pass metabolism lower than men, when given 10% or 40% but not 5% alcohol solutions [15].

Furthermore, ADH activity was demonstrated dependent not only on gender but also on age [16]. In social drinking male, gastric ADH activity is at the top level at 20-40 yrs and decreases with ageing until, at 61-80 yrs, it becomes approximately the half of 20-40 yrs activity. In women, gastric ADH activity is at the lowest level at 20-40 yrs, reaches the top at 41-60 yrs and decreases at 61-80 yrs. Thus, significant gender-related differences in gastric ADH activity appear during lifetime and with a trend to flatten in old age. The critical point is just at young age (20-40 yrs), when the gender difference is at the top level and females are more exposed to alcohol effects. The age 20-40 yrs is the age of fertility, and the increased risk just during the reproductive years results in an improved risk of fetal exposure and damage. BAC levels are related to an impairment of cognitive and psychomotor performance that increases together with BAC levels [17]; thus, the higher BAC levels result in a noticeable risk for female performance.

Drinking females may outstrip BAC legal limits for car driving easily (Italy: 50 mg/100 ml of blood) and undergo legal consequences, as well as may be victims of accident and violence. Recent investigations indicate that alcoholic brain damage is much more common than previously suspected and the potential sex-related difference in the susceptibility to the detrimental effects of chronic alcohol exposure on subsequent behaviour (cognitive aspects are mainly impaired) are investigated.

Even physiologic hormonal pattern have a role in vulnerability of female organism to alcohol intake.

Different reactions to alcohol intake may be observed in different phases of the menstrual cycle [18] and the mechanisms involving the estrogens in inflammatory processes are suggested to explain the more rapid progress of liver damage in female [19]. Furthermore, in the reproductive years, the use of oral contraceptives may worsen alcoholic damage by hormonal activation of endotoxin-induced liver injury [20]. Epidemiological and experimental data demonstrate that alcohol drinking may lead to female fertility impairment, [21] and increase of the risk of breast cancer [22-24].

Moreover, the teratogenic effect of alcohol is to be considered. Alcohol passes through placenta and reaches the fetus that is no tolerant to ethanol. Ethanol may interfere with fetal development causing abortion, fetal death, premature birth, low birth weight, abnormalities in mental and physical development, somatic alterations. The teratogenic effects of alcohol are globally defined as fetal alcohol spectrum disorders (FASD) and the fetal alcohol syndrome (FAS) is the worst occurrence. Fetal damage is not dose-related and may occur even at low levels of maternal alcohol intake, above all if ingested in the early pregnancy. Fetal alcohol damage is not reversible, and may be preventable avoiding any maternal alcohol intake during pregnancy.

FEMALE DRINKING AT DIFFERENT AGES

Adolescent age is a period of great physical changes and brain modifications, leading to a wide range of effects on the psychological functions and behaviour of adolescent [25]. Risk-taking behaviour, as experimenting with alcohol and other drugs (novelty seeking), is more common at this age. The volume of the hippocampus, a brain region important for learning and memory, in adolescents with alcohol-related problems is significantly smaller than in controls [26]. Women may be more susceptible than men to modifications of brain regions [27]. During adolescence dramatic changes in hormone levels and patterns occur and gender differences in the body's hormonal response to stress emerge. Girls may be especially vulnerable to stress [28, 29] and the levels of perceived stress may be a predictor of alcohol and other drug use [30]. Severe depression too plays a role amplifying the impact of substance use and abuse. Because females of all ages appear to be at greater risk for affective disorders, alcohol use/abuse among adolescent females is particularly worrisome. Animal studies suggest that alcohol may affect the adolescents differently than adults and the adolescents appear to be more sensitive to alcohol-induced damage in certain types of memory [31, 32]. Gender differences regarding the alcohol effects on developing adolescent brain and other body systems need further research. However, several evidences suggest that the younger a person begins to drink, the more likely he or she will develop alcohol problems later in life [33].

In the reproductive years, heavy drinking has been shown to disrupt normal menstrual cycling and reproductive function, leading to infertility and increased risk for spontaneous abortion [34, 35]. Maternal drinking during pregnancy may generate impaired fetal growth and development with a wide range of effects on exposed offspring (hyperactivity, attention problems, learning and memory deficits, and problems with social and emotional development).

These problems usually emerge at school age, and are globally defined as fetal alcohol spectrum disorders (FASD). The most serious consequence of maternal drinking during pregnancy is the fetal alcohol syndrome (FAS), that shows a distinctive set of facial anomalies, growth retardation, and significant learning and/or behavioural problems. A generalized deficit in complex information processing constitutes the central feature of the cognitive-behavioral phenotype of FASD [36].

As no safe threshold of alcohol use during pregnancy has been established, women who are pregnant, planning a pregnancy, or at risk for pregnancy should not drink alcohol at all: even children exposed to low levels of alcohol in their prenatal life may exhibit learning and behavioral problems [37].

Some evidences suggest that alcohol consumption may increase the risk of breast cancer. Moreover, even low levels of drinking may be a risk factor for women with family history of breast cancer [38, 39]. In middle-aged women, the use of hormone replacement therapy (HRT) during menopausal stage is a known risk factor for breast cancer and even moderate amounts of alcohol may increase significantly this risk [40].

In the last years, many topics about alcohol effects on different organs are under discussion. In postmenopausal women, alcohol consumption may affect several organs, like liver, brain, and gastrointestinal tract, directly; indirectly, altering the blood levels of sex steroids, may increase the risk for some diseases [41]. A debate matter is: may moderate alcohol drinking modulate a possible beneficial effect on health at this age? Many epidemiological evidences suggest that light-to-moderate alcohol consumption significantly reduces the risk of atherosclerosis in both genders by lowering the low-density lipoprotein (LDL), or "bad" cholesterol, increasing the high-density lipoprotein (HDL), or "good" cholesterol, and reducing blood clotting and the "stickiness" of platelets. On the contrary, heavy drinking can damage the heart. The problem is: what is the "safe" alcohol consumption for each individual?

A well-known problem of older age is osteoporosis, a skeletal disease characterized by low bone mass, increased bone fragility, and susceptibility to fracture [42, 43]. At menopause, the rate of bone loss increases significantly and some epidemiological studies suggest that light-to-moderate alcohol consumption may be associated with increased bone mineral density and decreased fracture risk in postmenopausal women [44-46]. However, heavy drinking may compromise bone health and increase the risk of osteoporosis, leading to

decreased bone density and impaired bone mechanical properties. These effects are mainly patent in young women, whose bones are still developing, but also chronic alcohol use in adulthood can harm bone health [47] in addition with other lifestyle factors, as tobacco smoking, that may increase the risk of osteoporosis and fractures [48].

Among elderly people, memory and brain function Alzheimer's disease (AD) is the most common form of dementia. It is characterized by progressive changes in cognitive ability, memory and mood. Women appear to be at greater risk than men for AD, also taking into account the women's longer life span [49]. Heavy alcohol drinking may increase the risk for AD in both genders, mainly in women, as they appear to be more vulnerable than men to alcohol-induced brain damage. However, moderate alcohol consumption doesn't seem to have negative effects on brain function, and moderate drinking could protect the blood vessels in the brain, as in the heart, against atherosclerosis. But the safe limits of alcohol intake are difficult to be defined because of individual variations in susceptibility to damage. The "optimum" amount of alcohol for each individual cannot reasonably be established.

ALCOHOL AND ENVIRONMENT

Heavy drinking is *per se* a risk factor for health, since it can trigger many pathological processes. Research on ethanol metabolism have established that alcohol is hepatotoxic, both because of secondary malnutrition and through metabolic disorders associated with ethanol oxidation. These effects are due to redox changes produced by the nicotinamide adenine dinucleotide NADH generated via the liver ADH pathway which in turn affects the metabolism of lipids, carbohydrates, proteins and purines. In addition to ADH, ethanol can be oxidized by liver microsomes by the ethanol-inducible cytochrome P450 (CYP2E1) which contributes to ethanol metabolism and tolerance and to the selective hepatic perivenular toxicity of various xenobiotics. This may explain the increased susceptibility of the heavy drinkers to the toxicity of industrial solvents, anesthetic agents, commonly prescribed drugs, chemical carcinogens and even nutritional factors such as vitamin A. The induction of the microsomal pathway contributes to increased acetaldehyde generation, with formation of protein adducts. This results in antibody production, enzyme inactivation, decreased DNA repair, and a striking impairment of the ability of the liver to utilize oxygen. Moreover, acetaldehyde promotes GSH depletion, free-radical-mediated toxicity and lipid peroxidation and increase hepatic collagen synthesis and accumulation [50].

Thus, alcohol drinking significantly enhances negative effects due to simultaneous exposure, professional or environmental, to other toxics. Probably never as in our days lifestyle and environment together play significant role for the health hazard of worldwide population. At present, alcohol drinking is usually considered in work places as a risk factor only for its acute effects on performance, mainly for activities such as pilots, car, bus drivers, industrial workers. There is a lack of data about organic damages due to professional (or environmental) exposure to toxics together with heavy drinking. Indeed, also the assessment of ethanol intake in toxicological studies is quite questionable: ethanol intake is estimated by self reported consumption, which resulted poorly reliable in several studies. Real at risk drinking should be identified by specific biological markers, as blood alcohol concentration (BAC), transaminases (AST and ALT), mean corpuscular volume (MCV), gamma-glutamil-transferase (GGT); alcoholism biomarkers may be a useful tool to evaluate the real alcohol consumption, but their values may be modified by the exposure of toxics. Thus, the classic biomarkers of alcoholism cannot fully discriminate between the effects of alcohol or some other toxics.

Dangerous lifestyle, such as smoking, drug abuse, alcoholism, may act synergically together with environmental toxics and promotes neurotoxicity, GABA system alteration, mitochondrial damage, impairment of immune system and teratogenic effects [51].

Chronic heavy drinking may be associated with an unbalance of some essential elements, such as iron, zinc, copper and selenium, because of an impairment of homeostatic mechanisms that in physiological conditions maintain these elements within the physiological limits. Levels of toxic metals may increase in chronic alcohol intoxication, because of a reduced availability or activity of regulatory essential nutrients and substances. Thus, concentrations of metals considered safe for general population can become unsafe for heavy drinkers. For example, lead is one of the most toxic metals and, in the last years, many limits for the use of this element were set to reduce the general exposure to this element. Nevertheless, the modern lifestyle produces many sources of exposition, continuously renewed, which cause a wide spread of lead; moreover, changes of population habit may trigger health risk situations, sometime ignored or underestimated. Most of ingested lead is rapidly excreted but, when the dose increases, relatively more is absorbed. Much of adsorbed lead is immobilized and incorporated in bone and hair, but some of it is concentrated in the liver with deleterious effects. Old and more recent studies have outlined that prenatal and postnatal development are compromised significantly by the presence of lead in the body: cognitive performance is affected, and even the risk of developing psychiatric diseases as schizophrenia is debated [52-54]. Since ethanol too have teratogenic effects, female alcoholics are a population heavily at risk for lead exposure, and their children will have a very high risk of neurobehavioral damage from lead and ethanol, since prenatal exposure to alcohol and lead intoxication seem affect the same brain functions. The developing brain is mainly vulnerable to the toxicants that may be responsible for learning disabilities and behavioural problems in children. It was demonstrated that lead exposure, like prenatal exposure to alcohol, affects cognitive performances

severely in children and may trigger antisocial behaviour in adolescence [55, 56].

Furthermore, other factors may concur to synergic effect of lead and ethanol. It is well known that tthiamine (vitamin B1) is an effective antidote against lead intoxication, but this vitamin results dramatically reduced in alcoholics, because of both malnutrition and impaired phosphorilation. Thiamin deficit is more severe in alcoholic women, resulting more exposed than men to side effects of lead [57].

Experimental data from animal models [58] demonstrated that co-exposure to lead and ethanol produced more elevation of blood zinc protoporphyrin and hepatic lipid peroxidation. Compared with the group treated with lead alone, lead-ethanol exposure lowered the concentration of blood and liver magnesium and calcium and increased the amount of lead in the blood, liver and brain. Chronic alcohol intake results in calcium and magnesium loss but co-exposure to lead and ethanol could result in more serious depletion of calcium and magnesium suggesting a synergism between alcohol consumption and lead poisoning. Results from another study [59] suggest that lead metabolism is modified by alcohol, and that heavy drinkers may be a risk population for saturnism.

The association of alcohol drinking with an increasing risk of certain types of cancer is a well known problem. Excess drinking can cause DNA damage by several mechanisms including increased cellular proliferation, oxidative stress, formation of lipid peroxidation products and related DNA adducts, inhibition of DNA repair.

The research focuses on the role of acetaldehyde, the metabolite formed from ethanol by the action of ADH and subsequently converted to acetate by aldehyde dehydrogenase (ALDH) [60].

The most recent studies have individuated the role of polyamines, natural compounds essential for cell growth, that reacts with acetaldehyde to trigger a series of reactions that damage DNA, an event that can lead to the development of cancer [61].

The polyamines facilitate the conversion of acetaldehyde into crotonaldehyde (CrA) that generates the DNA adduct, 1,N(2)-propano-2'-deoxyguanosine (PdG) called Cr-PdG. It was found that, under physiologically relevant conditions, polyamines stimulate the formation of Cr-PdG from acetaldehyde and dG or from acetaldehyde and DNA by directly reacting with acetaldehyde to generate CrA. The Cr-PdG adducts are also endogenous lesions apparently derived from lipid peroxidation. The adduct Cr-PdG is believed to be responsible for mutagenic and genotoxic effects [62]. But crotonaldehyde is not only an endogenous product but also a chemical present in the environment that is a powerful irritant, mainly for eyes and lungs, that may impair immune function and may cause cancers in animals [63, 64]. Crotonaldehyde is naturally present in food and is formed by the burning of fossil fuels (including waste gas from motors) and wood, cigarette smoke and cooking oils. Professional exposure may occur in chemical and war industry. Crotonaldehyde is produced mainly

as intermediate for sorbic acid production, and for the production of flavourings, solvents, substances of industrial interest, disinfestants for human, veterinary, domestic, and civil use, adhesives, inks and paints. The finding that polyamines may lead to the formation of endogenous crotonaldehyde and its DNA adducts, may be of great interest for understanding the mechanisms by which alcoholic beverage consumption increases the risk of cancer development. Furthermore, the possible role of both exogenous and endogenous crotonaldehyde in increasing cancer risk due to alcohol drinking and pollutants exposure, may stimulates further investigation about genetic factors, mainly those that influence DNA repair pathways, in relation to alcohol related risk of cancer and to individual susceptibility to alcohol drinking and environmental toxics.

FEMALE DRINKING AND BIOMARKERS

At present, no laboratory test alone can detect and quantify alcohol use lasting over a protracted period and distinguish between a single drinking episode and chronic alcohol use. Because biological markers currently in use may not be effective in screening for at risk alcohol use in a longer period, such as during pregnancy, clinicians most commonly use brief screening measures that rely on maternal self-reports to assess drinking pattern. Such screening measures have major disadvantages. One is that often is difficult for people to correctly recall their actual amount and frequency of alcohol intake. Furthermore, above all for women and pregnant women, the fear of punishment and disapproval for drinking alcohol can make them reluctant to reveal alcohol use and prenatal alcohol use, especially if heavy use. Supplementing self-reports with a carefully selected panel of biological markers as AST, ALT, MCV, GGT, would allow the earlier identification of women who are at risk for heavy drinking and the earlier monitoring of their drinking behaviour during pregnancy. A large number of patients seen in clinical practice have an underlying alcohol problem and there is a pressing need for accurate methods to objectively diagnose alcohol over-consumption. The problem is: how best to use biological markers to support the diagnosis of alcoholism [65]? Until few years ago, many longitudinal studies on alcohol dependence examined only male population. Women are poorly represented also in treatment studies even because they enter to alcohol facilities less than men. Thus, data from alcohol studies come essentially from males but results are often generalized to both sexes. This problem involves also alcohol biomarkers studies and only recently there is a greater attention to differences between sexes and gender studies are promoted. In fact it was demonstrated that gender has to be taken into account when the results of such a test are evaluated since significant interaction between gender and alcohol biomarkers was found [66]. In our previous studies about alcohol biomarkers, we verified the relevance of assessing actual drinking not only by a self-report questionnaire,

but also by the direct determination of BAC. This was demonstrated a good strategy for a correct evaluation of the diagnostic power of the biomarker on study. In the study about the mitochondrial isoenzyme of AST (mAST), we monitored a series of controls and on-treatment alcoholics, assessing simultaneously mAST and BAC. The results demonstrated mAST suitable to discriminate between controls and alcoholics; but the most relevant result was that mAST was suitable to discriminate between "BAC positive" actual drinkers (including self-claimed abstinent) and "BAC negative" actual abstinents. Furthermore mAST value increased or decreased in a short time (two days), in response to BAC level. Thus, acute alcohol consumption was demonstrated a significant, suggestive and until now inadequately examined factor in evaluating the diagnostic suitability of alcohol biomarkers [67].

In diagnostic evaluation, a debated problem is the usefulness of the evaluation of the area under the curve (AUC). Some authors show it as a good tool to create an algorithm and to increase the diagnostic accuracy of combined biomarkers above all when gender studies are considered [66]. In recent researches on thiamine (vitamin B1) and its esters, we found highly significant differences between alcoholics and control for thiamine (T) and thiamine difosphate (TDP or cocarboxylase) values, and no gender differences among the values in alcoholics. But, when the AUC of the ROC curves were evaluated, differences between alcoholic men and women were found and female AUC were significantly closer to 1, both for T and TDP [57, 68].

Experience clearly shows that particular attention may be due to gender differences when biological data are to be evaluated. In pregnant women, alcohol abuse biomarkers have to be carefully evaluated, because of the risk of fetal damage. FASD is a preventable cause of mental retardation and birth defects, and an early identification of at risk alcohol use may adverse fetal outcomes. The clinical laboratory can help to assess prenatal alcohol use [69] and can give a valuable contribution to prevent fetal damage. The clinical utility of biomarkers as blood/breath alcohol concentration, GGT, MCV, hemoglobin associated acetaldehyde (HAA) would be a mainstay in alcohol use detection, mainly in pregnancy where the presence of several positive markers should be correlated with at risk pregnancies [70]. Alcohol use during pregnancy is a significant public health problem. The health and social costs of prenatal alcohol use are very high. Some evidence indicates that even low dose drinking during pregnancy can cause adverse fetal effects but how this damage occurs is not fully understood. Because of alcohol adverse effects on the fetus, all women should be advised to abstain from drinking during pregnancy. Bio-tests that could identify women who continue to drink while pregnant would be invaluable to facilitate intervention for helping to stop alcohol drinking during pregnancy, to identify children at risk for alcohol associated birth effects and to monitor them from the birth for potential problems and, if needed, to facilitate their early approaches to special facilities.

Above all, the diagnosis of FASD should be established in at risk children before school age, to reduce mental health problems, school failure, antisocial behaviours and future problems with alcohol and other drugs. The intervention for mothers may help them to reduce their problem drinking, to enhance their ability to care for their children and to reduce the risk of alcohol exposure in their subsequent pregnancies. Biological samples for detecting drinking during pregnancy are traditionally neonatal or maternal urine and blood. More recently, other samples could be used after delivery to assess biomarkers of prenatal alcohol consumption, as amniotic fluid, cord blood, neonatal hair, placenta, breast milk, meconium and vernix (*i.e.* the cheese-like material that covers the skin of fetus). No single marker is sensitive and specific enough to be considered a gold-standard biomarker for prenatal alcohol use and panels of two or more markers may yeld greater sensitivity and specificity [71, 72]. Current biochemical markers are not as diagnostically effective in women as in men. Studies evaluating biomarkers in female are very limited and validated biomarkers with greater diagnostic sensitivity and specificity are needed, mainly to monitor pregnant women. In the last years, there is great interest in the study of fatty acid ethyl esters (FAEE) the metabolic products that result from the interaction between alcohol and fatty acids. FAEE can be detected in the cord blood, meconium and hair of children, and in other organs in adults [73, 74]. In the studies about thiamine, alcohol damage resulted higher in women than in men, confirming the so-called "telescoping effect" *i.e.* a more rapid progression of alcohol damage. Indeed, it was demonstrated that thiamine deficit has to be corrected in alcoholics, and above all in woman, since may be responsible of a worrisome worsening of the clinical situation of the patient. In drinking pregnant women, T deficit may be further increased by hyperemesis gravidarum with heavy consequences for woman health and with strong risk of damage for the central nervous system of the fetus [75]. So, the assessment of thiamine during pregnancy may be relevant, both as a marker of alcohol abuse and as an alert for the need of vitamin supplementation. More recent advances in the field of alcohol studies offer proteomics as new promises for developing biomarkers able to detect biological changes due to alcohol use and to distinguish between subjects currently drinking and true abstainers [76]. It was demonstrated that clusters of proteins can collectively distinguish between children with and without FAS and the aim is to monitor women at drinking risk. Development in proteomics would be a significant step in primary prevention of alcohol related prenatal damage.

Taken together, the results of many different studies outline the greater severity of alcohol-related damage in female, as well as the need of alcohol prevention programs especially targeted at women.

References

1. Meyerhoff DJ, Bode C, Nixon SJ, de Bruin EA, Bode JC, Seitz HK. Health risks of cronic moderate and heavy alcohol consumption: how much is too much? *Alcohol Clin Exp Res* 2005;29(7):1334-40.

2. Mancinelli R, Guiducci MS. La donna e l'alcol: vulnerabilità biologica? *Ann Ist Super Sanità* 2004;40(1):19-23.

3. National Institute on Alcohol and Alcohol Abuse (NIAAA). *Alcohol and woman*. Bethesda: NIAAA; 1990 (Alcohol Alert n.10: PH 290).

4. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Huges M. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the USA: results from the National Comorbidity Survey. *Arch Gen Psychiatry* 1994;51:8-19.

5. Substance Abuse and Mental Health Services Administration. *Summary of findings from the 1999 National Household Survey on Drug Abuse*. Rockville: US Department of Health and Human Services; 2000.

6. European Monitoring Centre for Drugs and Drug Addiction. *Male and female drug use: is the gap narrowing?* Lisbona: EMCDDA; 2005 (Drugnet Europe Issue 50 April-June 2005).

7. Warren KR, Foudin LL. Alcohol related birth defects, the past, present and future. *Alcohol Res Health* 2001;25(3):153-8.

8. Lanphear BP and Bearer CF. Biomarkers in paediatric research and practice. *Arch Dis Child* 2005;90:594-600.

9. Sasso GF, Mancinelli R, Attilia ML, Cavalieri G, Balducci G, Ceccanti M. *Markers biologici dell'abuso alcolico*. Roma: Edizioni SITAC; 2004.

10. Lieber CS. Metabolism of alcohol. *Clin Liver Dis* 2005;9(1):1-35.

11. Lieber CS. New concepts of the pathogenesis of alcoholic liver disease lead to novel treatments. *Curr Gastroenterol Rep* 2004 Feb;6(1):60-5.

12. Ely M, Hardy R ,Longford NT, Wadsworth MEJ. Gender differences in the relationship between alcohol consumption and drink problems are largely account for body water. *Alcohol Alcohol* 1999;34(6)894-902.

13. Goist K, Sutker P. Acute alcohol intoxication and body composition in women and men. *Pharmacol Biochem Behav* 1985;22(2):811-4.

14. Frezza M, Di Padova C, Pozzato G, Terpin M, Baraona E, Lieber CS. High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first pass metabolism. *N Engl J Med* 1990;322:95-9.

15. Baraona E, Abittan CS, Dohmen K, Moretti M, Pozzato G, Chayes ZW, Shaefer C, Lieber CS. Gender differences in pharmacokinetics of alcohol. *Alcohol Clin Exp Res* 2001;25(4):502-7.

16. Parlesak A, Billinger MHU, Bode C, Bode JC. Gastric alcohol dehydrogenase activity in man influence of gender, age, alcohol consumption and smoking in a Caucasian population. *Alcohol Alcohol* 2002;37(4):388-93.

17. Maruff P, Falleti MG, Collie A, Darby D, Mc Stephen M. Fatigue-related impairment in the speed, accuracy and variability of psychomotor performance:comparison with blood alcohol levels. *J Sleep Res* 2005;14(1):21-7.

18. Lammers SM. Do alcohol pharmacokinetics in women vary due to the menstrual cycle? *Addiction* 1995;90(1):23-30.

19. Day CP. Who gets alcoholic liver disease: nature or nurture? *J R Coll Physicians Lond* 2000;24:557-62.

20. Konno A, Enomoto N, Takei Y, Hirose M, Ikejma K, Sato N. Oral contraceptives worsen endotoxin induced liver injury in rats. *Alcohol Clin Exp Res* 2002;26(8):70S-74S.

21. Eggert J, Theobald H, Engfeldt P. Effects of alcohol consumption on female fertility during an 18-year period. *Fertil Steril* 2004;81(2):379-83.

22. Dumitrescu RG, Shields PG. The etiology of alcohol-induced breast cancer. *Alcohol* 2005;35:213-25.

23. Suzuki R, Ye W, Rylander-Rudqvist T. Saji S, Colditz GA, Wolk A. Alcohol and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status: a prospective cohort study. *J Natl Cancer Inst* 2005; 2;97(21):1601-8.

24. Onland-Moret NC, Peeters PH, van der Schow YT, Grobbeee DF, van Gils CH. Alcohol and endogenous sex steroid levels in postmenopausal women: a cross sectional study. *J Clin Endocrinol Metab* 2005;90(3):1414-9.

25. US Department of Health and Human Services, NHI, NIAAA. *Alcohol - an important women's health issue*. Bethesda: NIAAA; 2004 (Alcohol Alert n. 62, July 2004).

26. De Bellis MD, Clark DB, Beers SR, Soloff PH, Boring AM, Hall J, Kersh A, Keshavan MS. Hippocampal volume in adolescent-onset alcohol use disorders. *Am J Psychiatry* 2000;157(5):737-44.

27. Hommer D, Momenan R, Kaiser E, Rawlings R. Evidence for a gender-related effects of alcoholism on brain volumers. *Am J Psychiatry* 2001;158(2):198-204.

28. Ge X, Conger RD, Elder GH Jr. Pubertal transition, stressful life events, and the emergence of gender differences in adolescence depressive symptoms. *Dev Psychol* 2001;37(3):404-17.

29. Vik P, Brown SA. Life events and substance abuse during adolescence. In: Miller TW (Ed.) *Children of trauma*. Madison, CT: International Universities Press;1998.pp179-204.

30. Wagner EF. Delay of gratification, coping with stress and substance use in adolescence. *Exp Clin Psycopharmacol* 1993;1:27-43.

31. Silveri MM, Spear LP. Decrease sensitivity to the hypnotic effects of ethanol early in ontogeny. *Alcohol Clin Exp Res* 1998;22:670-6.

32. Markwiese BJ, Acheson SK, Levin ED, Wilson WA, Swartzwelder HS. Differential effects of ethanol on memory in adolescent and adult rats. *Alcohol Clin Exp Res* 1998;22:416-21.

33. Grant BF. The impact of a family history of alcoholism on the relationship between age at onset of alcohol use and DSM-IV alcohol dependence: Results of the National Longitudinal Alcohol Epidemiologic Survey. *Alcohol Health Res World* 1998;22:144-7.

34. Emanuele N, Emanuele MA. Alcohol alters critical hormonal balance. *Alcohol Health Res World* 1997;21(1):53-64.

35. Mello NK, Mendelson JH, Teoh SK. Overview of the effects of alcohol on the neuroendocrine function in women. In: Zakhari, S (Ed.) *Alcohol and the endocrine system*. Bethesda: NIAAA;1993 (National Institute on Alcohol Abuse and Alcoholism Research Monograph No.23.NIH Pub. No.93-3533: pp.139-69).

36. Riley EP, Mattson SN, Li TK, Jacobson SW, Coles CD, Kodituwakku PW, Adnams CM, Korkman MI. Neurobehavioral consequences of prenatal alcohol exposure: an international perspective. *Alcohol Clin Exp Res* 2003;27(2):362-73.

37. Department of Health and Human Services, Public Health Service. Surgeon General's advisory on alcohol and pregnancy. Washington: FDA;1981. *FDA Drug Bullettin* 11(2):9-10.

38. Vachon CM, Cerchan JR, Vierkant RA, Sellers TA. Investigation of an interaction of alcohol intake and family history on breast cancer risk in the Minnesota Breast Cancer Family Study. *Cancer* 2001;92(2):240-8.

39. Dorgan JF, Baer DJ, Albert PS, Judd JT, Brown ED, Corle DK, Campbell WS, Hartman TJ, Tejpar AA, Clevidence BA, Giffen CA, Chandler DW, Stanczyk FZ, Taylor PR. Serum hormones and the alcohol-breast cancer association in postmenopausal women. *J Natl Cancer Inst* 2001;93:710-51.

40. Nelson HD, Humphtey LL, Nygren P, *et al.* Postmenopausal hormone replacement therapy: Scientific review. *JAMA* 2002;288:872-81.

41. Register TC, Cline JM, and Shively CA. Health issues in postmeno-pausal women who drink. *Alcohol Res Health* 2002;26(4)299-307.

42. National Institutes of Health Osteoporosis and Related Bone Disease-National Resource Center. *Osteoporosis Overview*. Washington: NIH ORBD NC DC; 1999.

43. Edelson GW, Kleerekoper M. Bone mass, bone loss, and frac-tures. In: Matkovic V (Ed.) *Physical Medicine and Rehabilitation Clinics of North America*. Philadelphia: WB Saunders;1995,pp 455-64.

44. Bonnivk SL. *The Osteoporosis Handbook*. Dallas: Taylor Publishing; 1994.

45. Hansen MA, Overgaard K, Rus BJ, Christiansen C. Potential risk factors for development of postmenopausal osteoporosis-Examined over a 12 years period. *Osteopor Int* 1991;1(2):95-102.

46. Felson DT, Zhang YQ, Hannan MT, Kannel WB, Kiel DP. Alcohol intake and bone mineral density in elderly men and women- The Framingham Study. *Am J Epidemiol* 1995;142: 485-92.

47. Sampson HW. Alcohol and other factors affecting osteoporosis risk in women. *Alcohol Res Health* 2002;26(4):292-98.

48. Deng HW, Cheng WM, Conway T, Zhou Y, Davies KM, Stegman MR, Deng H, Recker RR. Determination of bone min-eral density of the hip and spine in human pedigrees by genetic and life-style factors. *Genet Epidemiol* 2000;19:160-77.

49. Sohrabji F. Neurodegeneration in women. *Alcohol Res Health* 2003;26(4):316-18.

50. Lieber CS. Biochemical factors in alcoholic liver disease. *Semin Liver Dis* 1993;13(2):136-53.

51. Campbell JD. Lifestyle, minerals and health. *Med Hypoptheses* 2001;57(5):521-31.

52. Bellinger D, Leviton A, Sloman J. Antecedents and correlates of improved cognitive performance in children exposed in utero to low level of lead. *Environ Health Perspect* 1990;89:5-11.

53. Dietrich KN, Berger OG, Succop PA, Hammond PB, Bornschein RL. The developmental consequences of low to moderate prenatal and postnatal lead exposure. Intellectual attainment in the Cincinnati Lead Study Cohort following school entry. *Neurotoxicol Teratol* 1993;15(1):37-44.

54. Opler MG, Susser ES. Fetal environment and schizophrenia. *Environ Health Perspect* 2005;113(9):1239-42.

55. Needleman HL, Schell A, Bellinger D, Leviton A, Allred EN. The long term effects of exposure to low doses of lead in childhood. An 11-years follow up report. *N Engl J Med* 1990;322:83-8.

56. Dietrich K, Ris M, Succop *et al*. Early exposure to lead and juvenile delinquency. *Neurotox Teratol* 2001;23:511-18.

57. Ceccanti M, Mancinelli R, Sasso GF, Allen JP, Binetti R, Mellini A, Attilia F, Toppo L, Attilia ML. Erythrocyte thiamine (Th) esters: a major factor of the alcohol withdrawal syndrome or a candidate marker for alcoholism itself? *Alcohol Alcohol* 2005;40(4):283-90.

58. Flora SJ, Kumar D, Sachan SR, Das Gupta S. Combined ex-posure to lead and ethanol on tissue concentration of essential metals and some biochemical indices in rat. *Biol Trace Elem Res* 1991;28(2):157-64.

59. Grilo Reina A, Lopez Artiguez M, Castilla L, Castro M, Garcia-Bragado F, Repetto M. Wine as source of lead contamination. Study in southern region of Sevilla. *Med Clin (Barc)* 1990;95(8):281-5.

60. Brooks PJ, Theruvathu JA. DNA adducts from acetaldehyde: implications for alcohol-related carcinogenesis. *Alcohol* 2005; 35(3):187-93.

61. Theruvathu JA, Jaruga P. Nath RG, Dizdaroglu M, Brooks PJ. Polyamines stimulate the formation of mutagenic 1,N2-pro-panodeoxyguanosine adducts from acetaldehyde *Nucleic Acid Res* 2005;33(11):3513-20.

62. Pan J, Chung FL. Formation of cyclic deoxyguanosine adducts from omega-3 and omega-6 polyinsaturated fatty acids under oxidative conditions. *Chem Res Toxicol* 2002;15:367-72.

63. Eder E, Budiawan. Cancer risk assessment for the environ-mental mutagen and carcinogen crotonaldehyde on the basis of TD50 and comparison with 1,N2-propanodeoxyguanosine ad-duct levels. *Cancer Epidemiol Biomarkers Prev* 2001;10:883-8.

64. Nath JE, Ocando JB, Guttenplan F, Chung L. 1,N2-propanode-oxyguanosine adducts: potential new markers of smoking in-duced DNA damage in human oral tissue. *Cancer Res* 1998;58: 581-4.

65. Schwan R, lbuisson E, Malet L, Loiseaux MN, Reynaud M, Schellenberg F, Brousse G, Llorca PM. The use of biologi-cal laboratory markers in the diagnosis of alcohol misuse: an evidence-based approach. *Drug Alcohol Depend* 2004;11;74(3): 273-9.

66. Chen J, Conigrave KM, Macaskill P, Whitfield JB. Combining CDT and GGT to increase diagnostic accuracy for problem drinking. *Alcohol Alcohol* 2003;38(6):574-82.

67. Macchia T, Mancinelli R, Gentili S, Ceccanti M, Devito R, Attilia ML, Taggi F. Mitocondrial aspartate aminotransferase isoenzyme: a biochemical marker for the clinical managements of alcoholics? *Clin Chim Acta* 1997;4;263(1):79-96.

68. Mancinelli R, Ceccanti M, Guiducci MS, Sasso GF, Sebastiani G, Attilia ML, Allen JP. Simultaneus liquid chromatography assess-ment of thiamine, thiamine monophosphate and thiamine diphos-phate in human erythrocytes: a study on alcoholics. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003;15;789(2):355-63.

69. Cook JD. Biochemical markers of alcohol use in pregnant women. *Clin Biochem* 2003;36(1):9-19.

70. Bearer CF, Stoler JM, Cook JD, Carpenter SJ. Biomarkers of alcohol use in pregnancy. *Alcohol Res Health* 2004/2005;281: 38-43.

71. Sarkola T, Eriksson CJ, Nemela O. MCV and GGT are superior to CDT and HAA in the follow-up of pregnant women with al-cohol abuse. *Acta Obstet Gynecol Scand* 2000;79:359-66.

72. Stoler JM, Huntington KS, Peterson CM. The prenatal detection of significant alcohol exposure with maternal blood markers. *J Pediatr* 1998;133:346-52.

73. Bearer CF, Jacobson JL, Jacobson SW. Validation of a new biomarker of fetal exposure to alcohol. *J Pediatr* 2003;143(4): 463-9.

74. Chan D, Caprara D, Blanchette P, Klein J, Koren G. Recent developments in meconium and hair testing methods for the confirmation of gestational exposure to alcohol and tobacco use. *Clin Biochem* 2004;37(6):429-38.

75. Ba A, Seri BV, Aka KJ, Glin L, Tako A. Comparative effects of developmental thiamine deficiencies on the morphometry of the CA3 pyramidal cells. *Neurotoxicol Teratol* 1999;21(5):579-86.

76. Neuhold LA, Gud QM, Alper J. High-throughput proteomics for alcohol research. *Alcohol Clin Exp Res* 2004;28:203-10.