Urinary Hydrogen Peroxide as Biomarker 13

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Abstract

Hydrogen peroxide can be found in human urine, not only in persons with diseases, but also in healthy individuals. In the body, hydrogen peroxide plays dual roles: on the one hand, it can produce more dangerous reactive oxygen species – hydroxyl radicals, which cause damage to the cells; on the other hand, it is considered as "a signaling molecule" to regulate the cellular processes. In this chapter, we will review the literature linking H_2O_2 excretion in urine of healthy individuals and of persons with diseases and present the available methods that have been used for detection of urinary H_2O_2 in human. We also give a brief overview on the association of urinary levels of H_2O_2 with other oxidative stress biomarkers and discuss the possibility whether urinary H_2O_2 could be a useful biomarker for the assessment of the oxidative status in human and for the prediction of the pathogenesis and progression of diseases.

Definitions of Words and Terms

Acatalasemic Mouse A mouse strain with lower catalase activities in hemolysate and tissues compared to that of wild type. A point mutation from glutamine to histidine (CAG → CAT) at amino acid 11 of catalase gene has been identified in acatalasemic mouse.

AOPP AOPP is an abbreviation for advanced oxidation protein products that can trigger the oxidative burst in neutrophils and monocytes. They are considered as inflammatory mediators.

ARDS ARDS is an abbreviation for adult respiratory distress syndrome which leads to insufficient oxygen levels in blood, and the cells cannot function well.

MDA MDA is an abbreviation for malondialdehyde, an end product of oxidative damage to lipids. It is a biomarker for oxidative stress.

Odds ratio (OR) Odds ratio is a measure of strength of association between two variables or sometimes between exposure and outcome. It is widely used to analyze data of clinical and epidemiological researches.

Introduction

Hydrogen peroxide (H_2O_2), a by-product of oxidative metabolism, is produced by superoxide dismutases (SOD) – catalyzed dismutation of superoxide radical ($O_2\cdot^-$) and some enzyme systems in vivo such as D-amino acid oxidases, monoamine oxidases, urate oxidase, glucose oxidase, and xanthine oxidase (Lynch and Fridovich 1979; Naqui et al. 1986; Strolin and Tipton 1998; Halliwell and Gutteride

2007; Veal et al. 2007). Hydrogen peroxide is considered one of the reactive oxygen species (ROS); although H_2O_2 itself is chemically less reactive, it is able to diffuse across the cell membrane and form other highly reactive intermediates like hydroxyl radical ($OH^{\cdot}$) in the presence of trace amounts of iron or copper (Halliwell and Gutteride 2007). If once $OH^{\cdot}$ are generated, they can attack the biological molecules such as DNA, protein, and lipid close to the site of their generation and lead to cell damage and subsequent development of diseases (Sun 1990; Halliwell and Gutteride 2007).

On the other hand, H_2O_2 is also considered an inter- and intracellular signaling molecule acting as "second messengers" to regulate the cellular processes in signal transduction cascades such as mitogen-activated protein (MAP) kinase and nuclear factor-κB (NF-κB) signaling pathways (Schreck et al. 1991; Wang et al. 1998; Allen and Tresini 2000; Wood et al. 2003; Veal et al. 2007). However, it is unclear under what conditions a useful signal is switched over to a harmful oxidant and vice versa, but some researchers have suggested the cytotoxicity induced by H_2O_2 at levels below 20–50 μM is "limited" (Halliwell et al. 2000, p. 10).

More than 20 years ago, Varma and Devamanoharan (1990) were the first to investigate whether human urine contains H_2O_2; they have found H_2O_2 with concentrations which ranged from 26 to 249 μM is excreted in human urine, and thereafter many works have confirmed the presence of H_2O_2 in urine of adults, adolescents, and newborn infants (Kuge et al. 1999; Long et al. 1999b; Laborie et al. 2000; Long and Halliwell 2000; Hiramoto et al. 2002; Kirschbaum 2002; Yuen and Benzie 2003; Chandramathi et al. 2009a, b; Chatterjee and Chen 2012; Sato et al. 2013). Investigation of the possibility whether urinary H_2O_2 can serve as a biomarker of oxidative stress in human has become a subject of scientific interest because of its noninvasive character of sample collection and simple quantification methods.

In this chapter, we will review the literature linking H_2O_2 excretion in urine of healthy individuals and of persons with diseases and present the available methods that have been used for detection of urinary H_2O_2 in human. We also give a brief overview on the association of urinary levels of H_2O_2 with other oxidative stress biomarkers and discuss the possibility whether urinary H_2O_2 could be a useful biomarker of oxidative stress in human.

Urinary H_2O_2 in Healthy Population

Table 1 summarizes a number of studies that have measured urinary H_2O_2 levels in humans. Even healthy persons excrete considerable amount of urinary H_2O_2 with great variation among individuals (Dunn and Curtis 1985; Long et al. 1999b; Yuen and Benzie 2003; Chatterjee and Chen 2012; Sato et al. 2013). Two studies (Varma and Devamanoharan 1990; Yuen and Benzie 2003) have shown no gender difference in excretion of urinary H_2O_2 among healthy individuals; the former examined 55 subjects (29 men, 26 women) 20–55 years old, and the latter examined 20 subjects (11 men, 9 women) 20–35 years old; whereas a recent study with a relatively

Table 1 Some examples of the detection of urinary H_2O_2 in human

Methods	Urine sample	Subjects	Results	References
[^{14}C]α-ketoglutarate decarboxylation assay	Fresh	Healthy individuals ($n = 20$; 11 males, 9 females)	Males: 106.4 ± 64.0 μM. Females: 88.6 ± 54.5 μM (mean ± SD)	Varma and Devamanoharan (1990)
[^{14}C]α-ketoglutarate decarboxylation assay	Urine samples (−40 °C)	Control ($n = 20$)	Control: 88 ± 4 μmol/l	Mathru et al. (1994)
		Patients with adult respiratory distress syndrome ($n = 7$)	Adult respiratory distress syndrome: 91 ± 3 μmol/l. Adult respiratory distress syndrome and sepsis: 97 ± 3 μmol/l	
		Patients with adult respiratory distress syndrome and sepsis ($n = 15$)		
FOX-2	Fresh	Healthy individuals ($n = 10$)	3.5–109.6 μM (30 min incub.); 1.1–112.6 μM (10 min incub.)	Long et al. (1999b)
O_2 electrode method	Fresh	Healthy individuals ($n = 22$)	Mean value: 33.7 μM (36.5 μM by FOX assay)	Long et al. (1999b)
FOX	Fasting urine; fresh	Healthy individuals ($n = 55$; 29 males, 26 females)	Males: 346 ± 266 μmol/mol Cr	Yuen and Benzie (2003)
			Females: 301 ± 200 μmol/mol Cr (mean ± SD)	
FOX-2	Fresh	Control ($n = 10$)	Control: 15 ± 9.8 μmol/l	Banerjee et al. (2003a)
		Cancer patients ($n = 25$) (10 carcinoma esophagus, 9 laryngeal carcinoma, 3 cervical carcinoma, and 3 breast carcinoma)	Cancer patients: 56.3 ± 3.9 μmol/l (mean ± SEM)	
FOX-2	Fresh	Control ($n = 15$)	Control: 20 ± 1.4 μM	Banerjee et al. (2004)
		Diabetic patients without complications ($n = 25$)	Diabetic patients: 42 ± 0.72 μM	

FOX-2	Urine samples (−70 °C) were analyzed within 24 h upon sample	Control ($n = 95$). Colorectal cancer patients ($n = 49$). Breast cancer patients ($n = 101$)	Control: 17.28 ± 0.83 μmol/l	Chandramathi et al. (2009a)
			Colorectal cancer patients: 23.23 ± 2.16 μmol/l. Breast cancer patients 17.65 ± 1.18 μmol/l (mean ± SEM)	
FOX-2	Fresh	Control ($n = 95$)	Control: 17.28 ± 0.83 μmol/l	Chandramathi et al. (2009b)
		Intestinal parasite infected ($n = 75$)	Intestinal parasite infected: 68.02 ± 7.23 μmol/l (mean ± SEM)	
FOX-2	Fasting urine (−80 °C)	Control ($n = 58$)	Control: 0.39 ± 0.40 μg/mg Cr.	Campos et al. (2011)
		Adolescents and adults with Down's syndrome ($n = 72$)	Persons with Down's syndrome 0.74 ± 0.69 μg/mg Cr (mean ± SD)	
FOX-1	Fasting urine (−80 °C)	Healthy individuals ($n = 766$; 323 males, 443 females)	Males: 5.05 ± 6.18 μmol/g Cr (Min.: 0.01, Max: 51.38)	Sato et al. (2013)
			Females: 6.10 ± 9.49 μmol/g Cr (Min.: 0.01, Max: 70.03) (mean ± SD)	
Electrochemical detection (carbon buckypaper)	Fresh	Healthy individuals ($n = 3$)	15.32–89.27 μM	Chatterjee and Chen (2012)

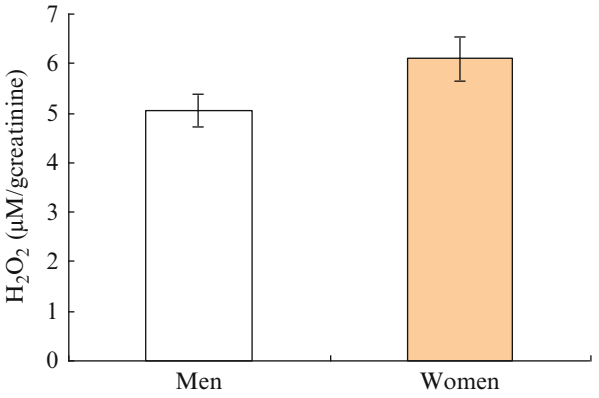

Fig. 1 The spot urinary H_2O_2 was evaluated in 766 healthy Japanese adults (323 men, 42.0 ± 10.2 years old; 443 women, 42.7 ± 10.9 years old). Data are expressed as mean and SEM (as bars) (Data are from Sato et al. (2013), with permission from the Publishers)

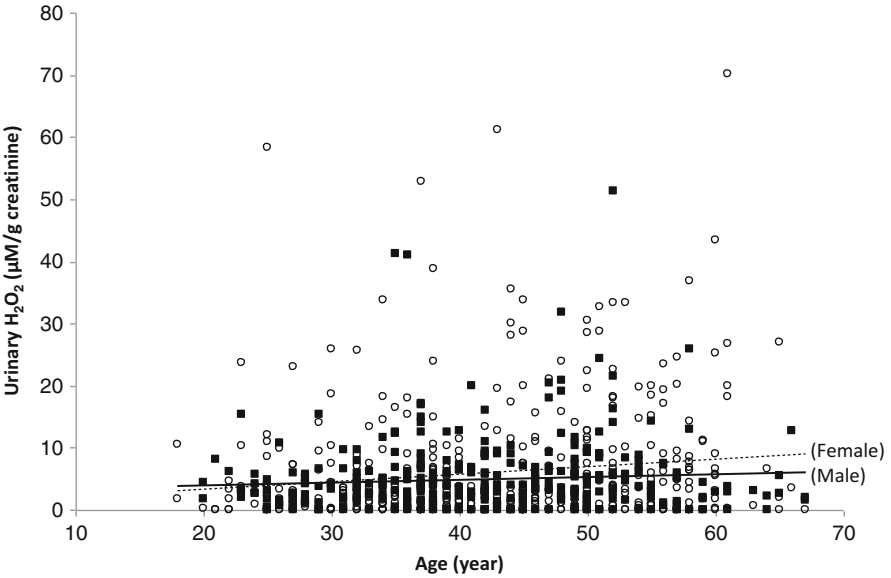

Fig. 2 The spot urinary H_2O_2 was evaluated in 766 healthy Japanese adults (Data are from Sato et al. 2013, unpublished)

large sample size in Japan has reported different results, it has found that there is a significant gender difference in mean value of urinary H_2O_2 (Fig. 1) (Sato et al. 2013). It is interesting that women over 50 years of age tend to excrete twofold amount of urinary H_2O_2 than those under 50 (Iwanaga et al. 2013), and there is an increased tendency in urinary H_2O_2 excretion in women over 50 years in comparison with that of the men (Fig. 2) (Sato et al. 2013), suggesting that urinary H_2O_2 levels increase among menopausal and postmenopausal women.

Urinary H_2O_2 and Lifestyle Parameters

Many studies have reported coffee drinkers excrete higher levels of H_2O_2 in urine (Long et al. 1999a; Hiramoto et al. 2002; Yuen and Benzie 2003; Chatterjee and Chen 2012), and salt loading also tends to increase mean excretion rate of urinary H_2O_2 that is highly correlated with sodium excretion, possibly by increased glomerular filtrate of sodium (Kuge et al. 1999), whereas green tea drinkers tend to excrete lower levels of H_2O_2 in urine (Halliwell et al. 2004). It is known that roasted coffee beans contain 1,2,4-hydroxyhydroquinone (HQQ), a compound that generates H_2O_2 (Hanham et al. 1983; Fujita et al. 1985; Hiramoto et al. 1998, 2001); when a person drinks coffee beverage, the body can absorb this compound and excrete it into the urine, and then autoxidation of HQQ in the urine can dose-dependently generate H_2O_2 (Rinkus and Taylor 1990; Tsuji et al. 1991; Halliwell et al. 2004; Chatterjee and Chen 2012).

Not only coffee drinkers but also smokers excrete a higher amount of H_2O_2 in urine; when the Chinese hamster ovary cells were exposed to urine fractions of smokers and coffee drinkers, the increased levels of urinary H_2O_2 are positively correlated with the ability of generating chromosome aberration (Dunn and Curtis 1985).

A relation between alcohol consumption and urinary H_2O_2 excretion was reported only in men under 50 years who drank three times or less per week, in which low urinary H_2O_2 excretion was observed after adjustment for biomedical parameters, markers, and lifestyle factors (Table 2) (Sato et al. 2013). More works need to be done to confirm the relation between alcohol consumption and urinary H_2O_2 excretion by age and sex.

Urinary excretion rate of H_2O_2 tends to be increased by exercise (Deskur et al. 1998; Kuge et al. 1999; Sato et al. 2013). Men under 50 years who exercised twice or less per week compared to those who did not exercise showed a more than doubled risk of urinary H_2O_2 excretion, even after controlling for the other lifestyle factors and biochemical parameters (Table 3), whereas no elevated urinary H_2O_2 was found in men under 50 years who exercised three times or more per week (Sato et al. 2013). It implies the levels of oxidative stress were higher in persons with low frequency of exercise per week (twice or less per week) than that of three times or more per week (Table 3). However, no information is available about whether those who exercise less frequently are more likely to have longer and more intense exercise each time. Exercise with higher intensity can result in the production of superoxide generation by mitochondria, xanthine oxidase, neutrophils, etc. (Sachdev and Davies 2008; Powers and Jackson 2008); it can also disturb reduced glutathione/oxidized glutathione (GSH/GSSG) redox balances in the liver, blood, and muscle tissues as well (Lew et al. 1985; Pyke et al. 1986; Gohil et al. 1988; Sastre et al. 1992). Some scientists have indicated that regular exercise can upregulate antioxidative enzymes like Mn-SOD, catalase, and glutathione peroxidase, in which the exercise-induced ROS are possibly involved in the activation of signaling pathways such as the NF-κB pathway, the transcriptional coactivators

Table 2 Association of alcohol consumption with odds ratio of urinary H_2O_2 according to alcohol consumption

	Alcohol consumption			
	No	3 times or less per week	4 times or more per week	p for trend
Male ($n = 323$)				
Age <50 ($n = 242$)				
Model 1[a]	1.00	0.45 (0.22–0.92)*	0.47 (0.23–0.97)*	0.042
Model 2[b]	1.00	0.47 (0.22–0.99)*	0.47 (0.21–1.05)	0.066
Age ≥50 ($n = 81$)				
Model 1[a]	1.00	1.38 (0.39–4.87)	1.25 (0.41–3.79)	0.693
Model 2[b]	1.00	1.88 (0.35–10.26)	1.51 (0.31–7.26)	0.606
Female ($n = 443$)				
Age <50 ($n = 304$)				
Model 1[a]	1.00	1.41 (0.87–2.28)	0.83 (0.39–1.77)	0.631
Model 2[b]	1.00	1.32 (0.79–2.21)	0.74 (0.32–1.70)	0.480
Age ≥50 ($n = 139$)				
Model 1[a]	1.00	1.69 (0.76–3.75)	1.23 (0.53–2.84)	0.635
Model 2[b]	1.00	1.48 (0.63–3.49)	1.05 (0.41–2.67)	0.918

Data are expressed as odds ratio (95 % CI)
Data are from Sato et al (2013), with permission from the Publishers
*$p < 0.05$. Data were analyzed by multiple logistic regression analysis
[a]Not adjusted
[b]Adjusted for BMI, hs-CRP, systolic blood pressure, RBC, WBC, ALT, TC, HbA1c, insulin, 8-OHdG, smoking, and exercise

PGC1α and PGC1 ß, and the transcriptional factor PPARγ in the cells (Ohno et al. 1986; Gomez-Cabrera et al. 2008; Ristow et al. 2009; Barbieri and Sestili 2012).

Association of Urinary H_2O_2 and Other Biomarkers

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is a product of oxidatively modified DNA base guanine (Halliwell and Gutteride 2007). Elevated urinary levels of 8-OHdG are considered an indicator of ROS-induced DNA damage in biological systems (Cooke et al. 2008). Sato et al. have observed the levels of fasting urinary H_2O_2 are positively correlated with the levels of urinary 8-OHdG in both men and women (2013); even after controlling for demographic, lifestyle, and clinical variables, a gender-stratified multiple logistic regression analysis demonstrated the excretion of H_2O_2 is much more likely to increase in the highest quartile of urinary 8-OHdG in a dose-dependent manner (Table 4). In addition, a study of 685 adults in Japan has suggested women over 50 years have elevated levels of urinary H_2O_2, 8-OHdG, serum ferritin, and hs-CRP in comparison with women under 50 (Iwanaga et al. 2013). Fifty years for a woman is the age to reach the menopause or postmenopause; the increased urinary H_2O_2 excretion is in parallel with the

Table 3 Association of exercise with odds ratio of urinary H_2O_2

	Exercise			
	No	2 times or less per week	3 times or more per week	p for trend
Male ($n = 323$)				
Age <50 ($n = 242$)				
Model 1[a]	1.00	1.90 (1.07–3.37)*	1.03 (0.52–2.06)	0.931
Model 2[b]	1.00	2.22 (1.17–4.20)*	1.29 (0.60–2.78)	0.521
Age ≥50 ($n = 81$)				
Model 1[a]	1.00	0.62 (0.22–1.73)	0.82 (0.28–2.46)	0.728
Model 2[b]	1.00	0.63 (0.18–2.22)	0.76 (0.18–3.30)	0.718
Female ($n = 443$)				
Age <50 ($n = 304$)				
Model 1[a]	1.00	1.32 (0.75–2.31)	1.39 (0.50–3.87)	0.525
Model 2[b]	1.00	1.35 (0.74–2.46)	1.36 (0.46–3.98)	0.575
Age ≥50 ($n = 139$)				
Model 1[a]	1.00	1.72 (0.76–3.90)	1.55 (0.68–3.55)	0.302
Model 2[b]	1.00	1.65 (0.67–4.08)	1.37 (0.56–3.32)	0.490

Data are expressed as odds ratio (95 % CI)
Data are from Sato et al (2013), with permission from the Publishers
*$p < 0.05$. Data were analyzed by multiple logistic regression analysis
[a]Not adjusted
[b]Adjusted for BMI, hs-CRP, systolic blood pressure, RBC, WBC, ALT, TC, HbA1c, insulin, 8-OHdG, smoking, and alcohol consumption

increased levels of markers of oxidative stress and inflammation in women over 50 years, possibly implying the contribution of excessive intracellular iron accumulation to the formation of oxidative stress and inflammatory response.

Table 5 shows that the relation between H_2O_2 in the urine and total cholesterol (TC) in the serum of healthy population seems to be affected by age after controlling for demographic, lifestyle, and biochemical variables, in which higher odds ratio of urinary H_2O_2 has been observed in the highest quartile of blood TC in men under 50 years old and in women under 50; whereas in men over 50, the tendency is opposite – the lower quartile of TC levels is paralleled by an increased H_2O_2 excretion in urine of the body. A recent cohort study of 12,740 adults in Korea has demonstrated that low serum cholesterol levels seem to increase mortality in men (Bae et al. 2012); however, it is unclear whether low serum cholesterol-related mortality is associated to the levels of H_2O_2 or other oxidative stress in the body.

Urinary H_2O_2 and Diseases

People may ask whether persons under pathological conditions would excrete more H_2O_2 in urine than healthy individuals. Many studies have found it is true of many diseases. We will give some examples based upon the published data.

Table 4 Association of urinary 8-OHdG concentrations with odds ratio of urinary H_2O_2

	Quartiles of 8-OHdG concentrations				p for trend
	Q1	Q2	Q3	Q4	
Male ($n = 323$)					
Age <50 ($n = 242$)					
Model 1[a]	1.00	1.10 (0.54–2.27)	1.76 (0.86–3.62)	2.16 (1.05–4.46)*	0.018
Model 2[b]	1.00	1.15 (0.53–2.47)	2.11 (0.96–4.64)	2.26 (1.01–5.03)*	0.019
Age ≥50 ($n = 81$)					
Model 1[a]	1.00	0.54 (0.14–2.07)	1.99 (0.57–6.90)	6.50 (1.59–26.51)**	0.002
Model 2[b]	1.00	0.71 (0.14–3.75)	4.37 (0.87–21.94)	12.33 (2.07–73.40)**	0.001
Female ($n = 443$)					
Age <50 ($n = 304$)					
Model 1[a]	1.00	1.14 (0.61–2.16)	1.20 (0.63–2.28)	2.07 (1.08–3.96)*	0.033
Model 2[b]	1.00	1.29 (0.65–2.56)	1.29 (0.64–2.60)	2.44 (1.19–5.01)*	0.021
Age ≥50 ($n = 139$)					
Model 1[a]	1.00	1.78 (0.69–4.60)	2.00 (0.77–5.18)	1.33 (0.51–3.46)	0.525
Model 2[b]	1.00	1.92 (0.68–5.42)	2.21 (0.79–6.23)	1.47 (0.53–4.10)	0.441

Data are expressed as odds ratio (95 % CI)
Data are from Sato et al (2013), with permission from the Publishers
*$p < 0.05$, **$p < 0.01$. Data were analyzed by multiple logistic regression analysis
[a]Not adjusted
[b]Adjusted for BMI, hs-CRP, systolic blood pressure, RBC, WBC, ALT, TC, HbA1c, insulin, smoking, alcohol consumption, and exercise

Urinary H_2O_2 and Cancer

The urinary excretion of H_2O_2 in cancer patients is two- to threefold higher (ten carcinoma esophagus, nine laryngeal carcinoma, three cervical carcinoma, and three breast carcinoma) than that of healthy controls ($n = 45$) (Table 1) (Banerjee et al. 2003a), in which other oxidative stress-related parameters like plasma hydroperoxide, erythrocyte malondialdehyde (MDA), plasma glutathione S-transferase (GST), erythrocyte GSH, and catalase increase among cancer patients as well (Banerjee et al. 2003a), and the levels of urinary H_2O_2 are significantly and positively correlated with these oxidative stress-related parameters in cancer patients.

Chandramathi et al. (2009a) have also reported that colorectal cancer patients excrete a significantly higher urinary H_2O_2 compared with that of healthy controls and breast cancer patients (Table 1).

Catalase is an enzyme that decomposes H_2O_2 into H_2O and O_2. In catalase-deficient acatalasemic mice, their mean catalase activities of mammary glands during pregnancy is 18.8 % of the wild-type mice (Ishii et al. 1996); increased incidences of spontaneous mammary tumors have been observed at 15 months after birth in acatalasemic mice, not in wild-type mice; in vitamin E-deprived acatalasemic mice, the cumulative incidence of spontaneous mammary tumors was higher compared with that in vitamin E-supplemented acatalasemic mice

Table 5 Association of total cholesterol levels with odds ratio of urinary H_2O_2

	Quartiles of total cholesterol concentrations				
	Q1	Q2	Q3	Q4	p for trend
Male ($n = 323$)					
Age <50 ($n = 242$)					
Model 1[a]	1.00	1.65 (0.80–3.41)	2.07 (1.01–4.23)*	2.12 (1.01–4.42)*	0.037
Model 2[b]	1.00	1.75 (0.81–3.79)	2.39 (1.09–5.25)*	2.57 (1.14–5.82)*	0.017
Age ≥50 ($n = 81$)					
Model 1[a]	1.00	0.31 (0.09–1.11)	0.25 (0.07–0.91)*	0.22 (0.06–0.82)*	0.025
Model 2[b]	1.00	0.29 (0.05–1.58)	0.11 (0.02–0.62)*	0.26 (0.04–1.63)*	0.078
Female ($n = 443$)					
Age <50 ($n = 304$)					
Model 1[a]	1.00	1.60 (0.83–3.07)	2.52 (1.33–4.79)**	2.55 (1.33–4.86)**	0.002
Model 2[b]	1.00	1.33 (0.67–2.63)	2.34 (1.19–4.58)*	2.42 (1.22–4.78)*	0.004
Age ≥50 ($n = 139$)					
Model 1[a]	1.00	0.65 (0.26–1.63)	1.17 (0.44–3.09)	0.67 (0.26–1.72)	0.675
Model 2[b]	1.00	0.72 (0.27–1.91)	1.17 (0.41–3.37)	0.60 (0.21–1.75)	0.548

Data are expressed as odds ratio (95 % CI)
Data are from Sato et al. (2013), with permission from the Publishers
*$p < 0.05$, **$p < 0.01$. Data were analyzed by multiple logistic regression analysis
[a]Not adjusted
[b]Adjusted for BMI, hs-CRP, systolic blood pressure, RBC, WBC, ALT, HbA1c, insulin, 8-OHdG, smoking, alcohol consumption, and exercise

(Ishii et al. 1996). These results may suggest the involvement of H_2O_2 or OH· generated from H_2O_2 in the mechanism of catalase-deficient mammary carcinogenesis.

Urinary H_2O_2 and Diabetes Mellitus

Superoxide dismutases are enzymes that accelerate the dismutation of $O_2^{\cdot -}$ into H_2O_2 and O_2. In comparison with the healthy individuals, the levels of urinary H_2O_2 excretion are twofold higher in diabetic patients, but the SOD activity in erythrocytes is about two-thirds of the healthy individuals (Banerjee et al. 2004; Drews et al. 2010), and erythrocyte catalase activity is also somewhat decreased in diabetic patients; these may suggest the contribution of endogenous $O_2^{\cdot -}$ to the increased H_2O_2 excretion in urine of diabetic patients. In addition, urinary H_2O_2 is inversely associated with fasting insulin in blood of healthy population (Szypowska and Burgering 2011; Sato et al. 2013). Pancreatic islets seem more sensitive to H_2O_2 because catalase gene expression is undetectable in pancreatic islets (Lenzen et al. 1996). Goth et al. have first reported a higher frequency of type 2 diabetes mellitus among Hungarian with catalase deficiency compared with those with normal catalase activity. Insufficient degradation of H_2O_2 may damage pancreatic β-cells and therefore increase the risk of diabetes (Goth and Eaton 2000; Pennathur

et al. 2001; Goth et al. 2004; Monnier 2001; McClung et al. 2004; Houstis et al. 2006; Ikemura et al. 2010; Goth and Nagy 2012). In alloxan-induced diabetic mouse model, several works have demonstrated increased incidence of hyperglycemia in acatalasemic mice compared with the wild type (Takemoto et al. 2009; Kikumoto et al. 2010; Kamimura et al. 2013).

Urinary H_2O_2 and Respiratory Distress Syndrome

In patients with adult respiratory distress syndrome (ARDS), not only expired (Kietzmann et al. 1993; Luczynska et al. 2003) but also urinary H_2O_2 levels (Mathru et al. 1994) are increased, particularly in patients with combined ARDS and sepsis, and urinary H_2O_2 levels are much higher (Table 1); the urinary levels of H_2O_2 in ARDS patients are nearly 70 % of those who suffer from both ARDS and sepsis. Changes in the urinary H_2O_2 levels may act as an indicator of improvement or aggravation in ARDS patients. It has been suggested that the possible sources of urinary H_2O_2 are some enzyme systems like NADPH oxidase and xanthine oxidase in phagocyte and endothelial cells as well as sepsis-induced bacteria (Oettinger et al. 1983; Mathru et al. 1994).

Urinary H_2O_2 and Intestinal Parasitic Infection

The urinary excretion of H_2O_2 is approximately fourfold higher in persons infected with intestinal parasites compared to the healthy individuals (Table 1); the increase of urinary H_2O_2 levels is in parallel with the levels of other oxidative stress-modulated products like MDA (an end product of lipid peroxidation) and AOPP (advanced oxidation protein products) in urine (Chandramathi et al. 2009b), and this may suggest a big amount of production of oxidative stress via phagocytosis-induced ROS in persons infected with intestinal parasites. Similar results have been proven in rats infected with intestinal parasite *Blastocystis hominis* (Chandramathi et al. 2010; El-Taweel et al. 2007).

Urinary H_2O_2 and Down's Syndrome

Down's syndrome (DS) is a genetic abnormality that causes intellectual and morphological retardation. Urinary H_2O_2 levels in persons with DS showed approximately twofold higher than that in controls (Table 1) (Campos et al. 2011). Studies have shown the Cu/Zn SOD activity among persons with DS is increased up to 50 % in comparison with that of the control group, while no alteration in catalase and reduced glutathione (GSH) activities has been observed (Gerli et al. 1990; Jovanovic et al. 1998). Such an imbalance between enzymes that generate or remove H_2O_2 possibly account for the elevated levels of H_2O_2, 8-OHdG, and MDA in urine of DS patients (Jovanovic et al. 1998; Campos et al. 2011).

In in vitro study, fetal DS neurons produce higher levels of ROS causing neuronal apoptosis, whereas treatment of cortical neurons from fetal DS and normal brain with catalase and vitamin E results in the prevention of DS neuron degeneration (Busciglio and Yankner 1995). Elevated production of ROS in neurons is possibly responsible for the intellectual retardation of DS patients.

Urinary Sample Collection and Storage

A slow increase of H_2O_2 level in urine has been observed when the urine is exposed to air (Hiramoto et al. 2002) or stored at room temperature over a few hours (Long et al. 1999b; Hiramoto et al. 2002; Yuen and Benzie 2003). Hydrogen peroxide levels in freshly voided urine at 4 °C are stable for up to 48 h (Yuen and Benzie 2003). Therefore, immediate measurement of H_2O_2 right after urine collection is of critical importance. The H_2O_2 levels are influenced by long-term storage of urine specimens at −80 °C (Yuen and Benzie 2003). Even if it is nearly impossible to immediately assay all samples for the population-based large studies, urinary H_2O_2 levels should be determined as early as possible when storing the samples at −80 °C to minimize artifactual generation of H_2O_2.

In addition, excretion of H_2O_2 in urine is affected by diet (Long and Halliwell 2000; Kuge et al. 1999; Halliwell et al. 2000, 2004; Hiramoto et al. 2002; Yuen and Benzie 2003; Chatterjee and Chen 2012); therefore, fasting urine specimen is essential for accurate evaluation of H_2O_2 levels in urine.

Methods for Detection of Urinary H_2O_2

Urinary H_2O_2 can be detected in healthy individuals (Varma and Devamanoharan 1990; Kuge et al. 1999; Long et al. 1999b; Laborie et al. 2000; Long and Halliwell 2000; Hiramoto et al 2002; Kirschbaum 2002; Yuen and Benzie 2003; Chatterjee and Chen 2012; Sato et al. 2013) as well as in persons under pathological conditions (Mathru et al. 1994; Banerjee et al. 2003b; Chandramathi et al. 2009b; Campos et al. 2011). In the literature, several methods have been reported for measurement of H_2O_2 in human urine. Among them, the FOX assay has become a widely used method of urinary H_2O_2 determination (Long et al. 1999b; Halliwell et al. 2000; Hiramoto et al. 2002; Banerjee 2003a, b; Banerjee et al. 2004; Yuen and Benzie 2003; Chandramathi et al. 2009a, b; Campos et al. 2011; Sato et al. 2013) because of its low cost and ease of use (Long et al. 1999b; Halliwell et al. 2000). The FOX assay involves oxidation of Fe^{2+} to Fe^{3+} by H_2O_2 and then subsequent formation of a chromophore (Fe^{3+}–xylenol orange complex) that can be measured at 560 nm (Nourooz-Zadeh et al. 1995). Earlier studies for detection of urinary H_2O_2 have mainly employed FOX-2 assay (Long et al. 1999b; Long and Halliwell 2000; Chandramathi et al. 2009a, b; Campos et al. 2011) that is usually used to measure plasma hydroperoxides level (Nourooz-Zadeh and Wolff 1994; Banerjee et al. 2003a) and seems to be not suitable for urine sample (Frei et al. 1988;

Banerjee et al. 2004), whereas a pH-adjusted FOX-1 assay (pH 1.7–1.8) in the presence of catalase has been suggested as a method with high sensitivity and specificity for urinary H_2O_2 detection (Banerjee et al. 2004). The intra-assay and inter-assay coefficients of variation (CV) have been reported as 4.3 % and 9.7 %, respectively (Sato et al. 2013).

The oxygen electrode assay is a simplest method for measurement of H_2O_2 in human urine (Long et al. 1999b; Halliwell et al. 2000); this method is based on reduction of O_2 at an O_2 electrode, and H_2O_2 levels are measured as O_2 release by adding catalase to the sample (Salazara et al. 2010). The drawback of this technique is less sensitive for urinary H_2O_2 detection (Halliwell and Gutteride 2007).

Varma and Devamanoharan (1990) first identified H_2O_2 excretion in human urine by using [^{14}C]α-ketoglutarate decarboxylation assay; this technique is based upon the ability of H_2O_2 to decarboxylate ^{14}C-labeled α-ketoglutarate to $^{14}CO_2$, and then $^{14}CO_2$ formation can be determined. The method is considered very sensitive for urinary H_2O_2 detection (Varma and Devamanoharan 1990; Kuge et al. 1999) although it uses radioactive technique.

Recently, nanomaterials have been used for determination of urinary H_2O_2 (Chatterjee and Chen 2012; Jiang et al. 2013). Chatterjee and Chen (2012) have employed titanium interfaced buckypaper biosensor for H_2O_2 determination in human urine; this technique is based upon "the co-immobilization of horseradish peroxidase and methylene blue on the functionalized carbon buckypaper." Jiang et al. (2013) recently developed a nonenzymatic biosensor based upon palladium/poly(3,4-ethylenedioxythiophene) nanocomposite-modified glassy carbon electrode for the detection of urinary H_2O_2 in human (Sanford et al. 2010). Although the approach using nanomaterials and electrocatalytic compounds for measurement of urinary H_2O_2 is considered very sensitive with good reproducibility (Chatterjee and Chen 2012; Jiang et al. 2013), these studies are conducted using small numbers of subjects, and more convincing evidence with increased sample size from human studies is needed.

For many years, the researchers have kept asking the question whether urinary H_2O_2 is capable of being used as a biomarker of oxidative stress in human. There is still uncertainty concerning the role of urinary H_2O_2 in assessment of oxidative status in population study because the excretion of urinary H_2O_2 varies among individuals and it can be affected by many factors. Factors like diet, smoking, and other lifestyle factors should be considered when designing a study on the association of urinary H_2O_2 with the diseases as well as when performing data analysis to avoid overestimation or underestimation of the association between urinary H_2O_2 excretion and the diseases. In the literature, most of the studies used a small sample-sized cross-sectional design to determine the relationship between urinary H_2O_2 excretion and the diseases because it is the easiest way to perform in human study. However, in order to examine the causal relations between urinary H_2O_2 levels and the generation of other oxidative biomarkers or between urinary H_2O_2 levels and disease development, it is worth performing prospective cohort studies (Albertini 1999; Bonassi et al. 2001), as well as examining the consistency of the findings

among studies, although such studies are costly and time-consuming and require a large number of participants.

Summary Points

- Urinary H_2O_2 can be detected in healthy individuals as well as in persons under pathological conditions.
- Healthy individuals excrete considerable amount of urinary H_2O_2 with great variation among individuals.
- In comparison with women under 50 years old, the urinary H_2O_2 excretion is twofold higher in women over 50, in parallel with the increased levels of markers of oxidative stress (urinary 8-OHdG, serum ferritin) and inflammation (hs-CRP).
- Coffee drinkers and smokers excrete relatively higher levels of H_2O_2, and green tea drinkers tend to excrete lower levels of H_2O_2 in urine.
- The levels of urinary H_2O_2 are positively correlated with that of urinary 8-OHdG, blood WBC, AST, ALT, TC, LDL-c, ferritin, age, and exercise, inversely correlated with that of blood insulin in healthy individuals.
- Excretion of urinary H_2O_2 is increased in patients with cancer, diabetes mellitus, hypertension, respiratory distress syndrome, intestinal parasitic infection, and Down's syndrome.
- Fasting urine specimen is essential for accurate evaluation of H_2O_2 levels in urine because excretion of H_2O_2 in urine is affected by diet.
- Urinary H_2O_2 levels should be determined as early as possible when storing the samples at $-80\ ^\circ C$ to minimize artifactual generation of H_2O_2.
- A pH-adjusted FOX-1 assay in the presence of catalase has been suggested as a method with high sensitivity and specificity for urinary H_2O_2 detection.

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