Association Among Obstructive Sleep Apnea Risk and Blood Chemistry Changes in the U.S. Population: National Health and Nutrition Examination Survey 2005 – 2008

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Abstract

INTRODUCTION

Sleep-Disordered breathing (SDB) is characterized by abnormalities in the respiratory pattern or ventilation during sleep. is a common disorder and previously estimated to affect up to 20% of the population. SDB is classified into 3 general categories: Obstructive sleep apnea (OSA), Central sleep apnea, and hypoventilation/hypoxemia syndromes. The present study analyzed the relationship between OSA risk and blood chemistry changes in a sample representative of the U.S. population surveyed during 2005-2008.

METHODS

Data obtained from the “Sleep Disorders” questionnaires from National Health and Nutrition Examination Survey 2005-2006 and 2007-2008 survey were analyzed. Sample was weighted to make results representative of the US Census civilian non-institutionalized population. This study used the modified STOP-Bang questionnaire to calculate disorder breathing risk score. Blood markers were used to determine specific abnormalities. OSA risk scores from 0 to 7 were analyzed using ordinal logistic regression analysis.

RESULTS

A total number of 10,352 subjects, who after weighting were representative of 205’811,176 U.S. non-institutionalized population, were eligible for the study. The modified STOP-Bang questionnaire results showed that 23.1 % of the population was in the high risk OSA group. Ordinal logistical regression analysis was statistically significant for glycohemoglobin, uric acid, and hematocrit.

CONCLUSIONS

Our result showed that in the U.S. Census civilian non-institutionalized population, higher OSA risk scores are associated with an increase in hematocrit, uric acid, and glycohemoglobin.

ABBREVIATIONS:
SDB: Sleep disordered breathing, NHANES: National health and nutrition examination survey, OSA: Obstructive sleep apnea, CSA: Central sleep apnea, PSG: Polysomnography, PSU: Primary sampling units, PPS: Probability proportional to size, BMI: Body mass index, BP: Blood pressure, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HDL: High density lipoproteins, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyl transferase, CO2: Carbon dioxide, BUN: Blood urea nitrogen, HPLC: High performance liquid chromatography, LDH: Lactate dehydrogenase, CRP: C-reactive protein, SD: Standard Deviation.

INTRODUCTION

Sleep-Disordered breathing (SDB) is characterized by abnormalities in the respiratory pattern or ventilation during sleep. SDB is a common disorder and previously estimated

to affect up to 20% of the U.S. population (1). In the presence of diurnal symptoms, SDB is classified into 3 general categories: Obstructive sleep apnea (OSA), Central sleep apnea (CSA), and hypoventilation/hypoxemia syndromes. Epidemiological studies have shown that symptomatic SDB is accompanied with increased risk of hypertension, myocardial infarction, stroke, heart failure, and motor vehicle accidents (2-5). Numerous studies had associated SDB with obesity, but the pathophysiological mechanisms are not well known.

The most widely used method to diagnose and measure SDB severity is a supervised overnight polysomnography (PSG) (6-8). Due to PSG costs several screening tools that could be used by primary care providers and sleep clinicians had been developed and validated (9-13).

The National Health and Nutrition Examination Survey (NHANES) is a study designed to assess the health and nutritional status of adults and children in the United States. The survey is unique in that it combines interviews and physical examinations and is conducted in households, with questionnaires administered to families, adults and children (14). In NHANES 2005-2006 and 2007-2008 a "Sleep Disorders" questionnaire was included. This questionnaire included questions related to sleeping habits, disorders and general productivity (15, 16).

The present study objective was to evaluate the relationship between OSA risk and physiological and metabolic blood chemistry changes in the U.S. Census civilian non-institutionalized population surveyed from 2005 to 2008 and with no medical diagnosis of any sleep disorder.

METHODS

Study Population

The NHANES 2005-2006 and 2007-2008 data was used in our study. NHANES sampling was done to a select group of participants who are representative of the civilian, non-institutionalized U.S. population. The sample does not include nursing home residents, members of the armed forces, institutionalized persons, or U.S. nationals living abroad. Samples were not obtained randomly (17). Sampling was done in four stages: Stage 1: Primary sampling units (PSUs) were selected, such as single counties or, in a few cases, groups of contiguous counties with probability proportional to a measure of size (PPS). Stage 2: PSUs were divided into segments (city blocks or equivalents). As with each PSU, sample segments were selected with PPS. Stage 3: Households within each segment were listed, and a sample was randomly obtained. In geographic areas where the proportion of age, ethnic, or income groups selected for oversampling is high, the probability of selection for those groups is greater than in other geographic areas. Stage 4: Individuals were chosen to participate in NHANES from a list of all persons residing in selected households. Individuals were drawn at random within designated age-sex-race/ethnicity screening sub-domains. Since NHANES is designed to sample larger numbers of certain subgroups of particular public health interest, oversampling is done to increase the reliability and precision of estimates of health status indicators for these population subgroups. For the period between 1999 and 2006 the oversampled subgroups were: Non-Hispanic black persons, Mexican-American persons, low-income white persons (beginning in 2000), persons aged 70 and over, and adolescents aged 12–19; and period between 2007 and 2010: Non-Hispanic black persons, Hispanic persons, low-income white persons, and persons aged 80 and over. Sometimes certain subgroups in the population are not oversampled, mainly due to the fact that it is either cost prohibitive and/or operationally not feasible to oversample certain groups in the population.

All subjects who satisfactory answered the "Sleep Disorders" questionnaires of NHANES 2005-2006 or 2007-2008 were included in the study. The exclusion criteria were an age younger than 20 years old, pregnancy and having a medical diagnosis of a SDB.

Obstructive Sleep Apnea Risk Scoring

OSA risk score was calculated using the STOP-Bang modified questionnaire (9, 11). STOP-Bang questionnaire consists of eight yes/no questions: snoring, tiredness/sleepiness during daytime, observed stop breathing during sleep, high blood pressure, BMI (> 35 kg/m2), age (over 50 years old), gender (male), and neck circumference (>40 cm). The present study used a modified seven questions STOP-Bang questionnaire which consisted in only the first seven STOP-Bang questions excluding neck circumference since NHANES physical examination did not include neck circumference measurements. One point was awarded for each yes question. OSA risk score estimated range was from 0 to 7. Subjects who scored 3 or less were considered low risk OSA, and more than 3 considered high risk OSA. Since the original eight question STOP-Bang OSA risk score was validated using the criteria of 3 or more

points for high risk with a range from 0 to 8 (11), in the present study and mainly due to the lack of one question, the classification was modified to more than 3 for high risk of OSA. This classification will prevent an overestimation in the number of subjects at high risk.

The NHANES 2005-2006 and 2007-2008 variables used for the calculation the OSA risk score were: Sex (riagendr), age (ridageyr), BMI (bmxbmi), "ever told you had high blood pressure" (bpq020), systolic blood pressure (mean of bpxsy2, bpxsy3, and bpxsy4), diastolic blood pressure (mean of bpxdi2, bpxdi3, and bpxdi4), snoring frequency (slq030), sleepiness (slq120), tiredness (slq110), and gasping (slq040). Systolic and diastolic blood pressures were measured up to 4 times during the same visits. The first value of each blood pressure measurement was excluded due to "white coat" effect and the following measurements were averaged separately. High blood pressure was considered positive if there was a medical story of high blood pressure or if at examination the systolic or diastolic blood pressures were equal or higher than 140 or 90 mmHg respectively.

Biochemistry

Blood laboratory markers were used as physiological surrogates. A detailed description of the laboratory methods used can be found in Appendix I and at the NHANES Laboratory Procedures Manual (18, 19). In brief, total Cholesterol was measured using a reaction catalyzed by cholesterol esterase that frees cholesterol and fatty acids. High Density Lipoproteins (HDL-Cholesterol) were measured directly in serum determined enzymatically. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in plasma were measured using an enzymatic activity rate method. Total Protein was measured using a timed rate biuret method. Albumin was measured combining it with Bromcresol Purple reagent. Alkaline Phosphatase (ALP) was measured using 2-Amino-2-Methyl-1-Propanol buffer. Gamma-Glutamyl Transferase (GGT) activity was measured from serum using and enzymatic reaction.. Bicarbonate was measured indirectly by quantifying the total carbon dioxide (CO2) level in serum or plasma. Blood Urea Nitrogen (BUN) was measured using an enzymatic conductivity rate method. Creatinine was measured using the Jaffe rate method (kinetic alkaline picrate). Creatinine values from NHANES 2005-2006 were standardized to NHANES 2007-2008 using the following formula: Cr (mg/dl)= -0.016 + 0.978 X (NHANES 05-06 serum Cr, mg/dl) as recommended by CDC (20). Glucose was measured by the oxygen rate method. Glycohemoglobin was measured directly using HPLC (High-performance liquid chromatography) for fraction separation and the HbA1c fraction flow measured by changes in absorbance at 415 nm which is proportional to its concentration. Lactate Dehydrogenase (LDH) was measured using an enzymatic activity rate method which reduces NAD to NADH. Uric acid was measured by oxidation that generates hydrogen peroxide. C-Reactive protein (CRP) was measured by latex-enhanced nephelometry.

Statistical analysis

Data from 2-year cycles NHANES 2005-2006 and 2007-2008 were combined. Sample weights were constructed to allow the population analyzed be representative of the U.S. civilian non-institutionalized population at the midpoint of the survey period (21, 22). The formula used for constructing weights was the one recommended by NHANES: MEC4YR = (0.5) X (WTMEC2YR) where WTMEC2YR is the Full Sample 2 Year MEC (mobile examination center) Exam Weight and MEC4YR the new constructed weight for analyzing the two 2-year cycles 2005-2006 and 2007-2008 (23). To determine the relationship between OSA risk score and different blood markers including physical examination variables means and proportions were analyzed using regression models and Wald test for surveys. In addition OSA risk scores whole distribution from 0 to 7 was analyzed using ordinal logistic regression analysis. This type of analysis allowed considering each OSA risk score independently and to look for a trend instead of grouping them in two groups as previously done. Moreover using the real OSA risk score prevents using the pre defined low and high criteria (3 or less or more than 3 respectively) which has not been validated in the US Census civilian non-institutionalized population. Since BMI, age and gender are usual confounders when analyzing biochemical and body measurements, comparison analysis were done adjusting for these three variables. BMI and age were used as continuous variables. The NHANES variables used to calculate OSA risk score and for the analysis of blood chemistry changes data were obtained from the following sources: a) demographics: age and gender; b) medical examination: body mass index (BMI), systolic and diastolic blood pressure; and c) serum laboratory questionnaires and tests: total cholesterol, HDL, ALT, AST, ALP, GGT, glucose, glycohemoglobin, total proteins, albumin, creatinine, BUN,

urinary albumin, uric acid, LDH, bicarbonate, CRP, and hematocrit. Data was analyzed using the statistical package STATA/IC 11.2 for Windows software (StataCorp LP, College Station, TX).

RESULTS

A total of 12,658 subjects answered the "Sleep Disorders" questionnaire NHANES 2005-2006 and 2007-2008. After considering the exclusion criteria previously described, a total number of 10,352 subjects were eligible for the study and after weighting were representative of 205'811,176 U.S. non-institutionalized population. The characteristics of these selected subjects are shown in Table 1.

Table 1

Characteristics of the population in the study

Weighted to U.S. Population*	Mean	Standard Deviation
Male %	49.2	-
Female%	50.8	-
Age (years)	47.0	16.7
BMI (kg/m²)	28.7	6.5
Total cholesterol (mg/dl)	198.1	41.1
HDL (mg/dl)	52.8	15.9
Alanine aminotransferase (U/L)	26.0	19.1
Aspartate aminotransferase (U/L)	25.7	14.1
Alkaline phosphatase (U/L)	68.3	23.3
Gamma glutamyl transferase (U/L)	29.7	44.5
Total serum protein (g/dl)	7.11	0.44
Serum albumin (g/dl)	4.25	0.32
Glucose (mg/dl)	98.9	33.3
Glycohemoglobin (%)	5.53	0.88
Urinary albumin (µg/ml)	37.5	337.5
Creatinine (mg/dl)	0.88	0.30
BUN (mg/dl)	12.9	5.3
Uric acid (mg/dl)	5.47	1.38
Lactate dehydrogenase (U/L)	129.5	26.7
Bicarbonate (mmol/L)	24.8	2.2
C-reactive protein (mg/dl)	0.41	0.82
Hematocrit (%)	42.2	4.2
Systolic blood pressure (mmHg)	121.8	17.1
Diastolic blood pressure (mmHg)	70.9	11.5

* Population size of 189'033,535 – 205'811,176

The male female ratio was 49.2/50.8, mean age was 47 with a SD of 16.7 years, and mean BMI of 28.7 with a SD of 6.5 kg/m2 within to the range of overweight. Total cholesterol mean value was 198.1 with a SD of 41.1 mg/dl, which is closer to the 200 mg/dl upper limit of desirable level. HDL, AST, ALT, ALP, GGT, total protein, albumin, glucose, glycohemoglobin, urinary albumin, creatinine, BUN, uric acid, LDH, bicarbonate, C-reactive protein, and hematocrit all within normal physiological values. Systolic blood pressure was 121.8 with a SD of 17.1 mmHg which is in the pre-hypertension range, and diastolic blood pressure was within normal values.

Low and high risk OSA population were determined using the modified STOP-BANG questionnaire. Distributions are shown in Table 2.

Table 2

Population in percentage with low and high risk of SDB based Modified STOP-Bang.

SDB Risk		Modified STOP-BANG*
Weighted to U.S. Population	%	95% CI
Low (Score≤3)	76.9	75.2 – 78.5
High (Score>3)	23.1	21.4 – 24.7

* Population size of 167'904,135

The modified STOP-Bang questionnaire results showed that 23.1 % of the population was in the high risk OSA group. Responses of the modified STOP-Bang questionnaire in high risk of OSA population are shown in Table 3.

Table 3

Modified STOP-Bang questionnaire answers distribution in High Risk of OSA

Modified STOP-BANG Question*	Percentage	
	No	Yes
Snoring at least 3 nights per week	11.1	88.9
Tiredness/Sleepiness during daytime	48.6	51.4
Observed stop breathing during sleep	42.9	57.1
High blood pressure	23.4	76.6
BMI > 35 kg/m²	65.0	35.0
Age > 50 years old	32.1	67.9
Male	29.9	70.1

* Population size of 38'754,959

The most frequent positive answer in the modified STOP-Bang questionnaire was the snoring frequency question, where 88.9 % of the population reported a snoring frequency of at least 3 nights per week. The second most frequent finding was high blood pressure with 76.6 %.

Comparison of blood chemistry values between low and high risk OSA based on modified STOP-Bang questionnaire dichotomize classification are shown in Table 4.

Table 4

Characteristics of High and Low Sleep Disorder Breathing risk by STOP-BANG questionnaire

OSA Risk Weighted to U.S. Population	Low* (Score ≤3)		High** (Score >3)	
	Mean	Standard Error	Mean	Standard Error
Male (%)	44.1	-	70.1	-
Female (%)	55.9	-	29.9	-
Age (years)	43.7	0.42	55.2	0.54
BMI (kg/m²)	27.4	0.11	32.6	0.21
Total Cholesterol (mg/dl)	198.0	0.56	197.5	1.38
Serum HDL (mg/dl)	54.0	0.35	48.6	0.46
Serum Alanine aminotransferase (U/L)	25.3	0.29	29.1	0.60
Serum Aspartate aminotransferase (U/L)	25.3	0.26	27.1	0.37
Serum Alkaline phosphatase (U/L)	66.7	0.57	73.0	0.67
Gamma glutamyl transferase (U/L)	26.2	0.57	40.6	2.22
Total serum protein (g/dl)	7.12	0.01	7.0	0.01
Serum albumin (g/dl)	4.27	0.01	4.2	0.01
Serum Glucose (mg/dl)	95.5	0.52	107.9	1.15
Glycohemoglobin (%)	5.42	0.01	5.82	0.03
Urinary albumin (µg/ml)	30.0	3.96	61.6	6.02
Serum Creatinine (mg/dl)	0.86	0.01	0.97	0.01
Blood urea nitrogen (mg/dl)	12.3	0.11	14.3	0.24
Serum uric acid (mg/dl)	5.29	0.02	6.06	0.04
Serum Lactate dehydrogenase (U/L)	127.5	0.43	134.5	0.67
Serum bicarbonate (mmol/L)	24.7	0.08	24.9	0.08
C-reactive protein (mg/dl)	0.35	0.01	0.54	0.02
Hematocrit (%)	42.0	0.17	43.1	0.20
Systolic blood pressure (mmHg)	119.0	0.28	129.0	0.54
Diastolic blood pressure (mmHg)	70.2	0.27	73.7	0.40

* Population size of 121'760,042 - 129'149,176

The P values shown were calculated after adjusting for BMI, age, and gender. The ordinal logistic regression analysis of OSA risk score using the modified STOP-Bang score from 0 to 7 is shown in Table 5.

Table 5

Odds Ratios of blood chemistry variables for Sleep Disorder Breathing risk score determined by the modified STOP-Bang questionnaire of U.S. non-institutionalized adults between 2005 and 2008.

Weighted to U.S. Population*	OSA risk score (from 0 to 7)**		
	Odds Ratio	95% CI	P Value
Serum Alkaline phosphatase (U/L)	1.003	1.001 - 1.006	0.004
Gamma glutamyl transferase (U/L)	1.002	1.000 - 1.004	0.026
Serum Glucose (mg/dl)	0.998	0.996 - 1.001	0.307
Glycohemoglobin (%)	1.219	1.108 – 1.341	<0.001
Serum Creatinine (mg/dl)	1.082	0.903 – 1.297	0.379
Serum uric acid (mg/dl)	1.099	1.052 – 1.148	<0.001
Hematocrit (%)	1.025	1.011 - 1.039	0.001

* Population size of 159'083,017

**Odds Ratio adjusted to BMI, age and gender. Odds ratio was calculated using the whole OSA risk score distribution from 0 to 7 by ordinal logistic analysis

Odds ratios of ALP (1.0), GGT (1.0), glycohemoglobin (1.2), uric acid (1.09), and hematocrit (1.02) showed statistical significance. All odds ratios shown were adjusted by BMI, age, and gender.

DISCUSSION

To our knowledge this is the first study analyzing OSA risk score in U.S. Census civilian non-institutionalized population with no medical diagnosis of a sleep disorder. Previous estimations have been done using non-representative samples of the U.S. population. In addition this study has used a simple and previously developed questionnaire that measures the risk of OSA, a tool available to every health care provider. STOP-Bang questionnaire was developed with a population of patients who where scheduled to undergo elective surgery and validated with their respective standard PSG. In subjects with a clinical diagnosis of mild, moderate or severe OSA, STOP-Bang showed a sensitivity of 83.6%, 92.9%, and 100% respectively and a PPV (predictive positive value) of 81%, 51.6%, and 31% respectively (9). Our study showed a OSA risk population prevalence of 23.1%, which is very close to the 20 % previously estimated (1).

Ordinal logistical regression analysis of OSA risk score distribution calculated using the modified STOP-Bang questionnaire showed ORs statistically significant for glycohemoglobin, uric acid, ALP, GGT, and hematocrit after adjusting for BMI, age, and gender. Our study found that for a one unit increase in glycohemoglobin, the odds of an increase in OSA risk score is 1.21 or a 21.9 % score increase independently of BMI, age, or gender. Previous association between an increase in glycohemoglobin and symptomatic OSA independent of obesity in non-diabetic population has been reported (23). Moreover, several studies have demonstrated the association between OSA and glucose intolerance (24, 25). It is possible that U.S. population with higher OSA risk scores could be experiencing changes related to glucose and insulin response.

About uric acid analysis, we found that for a one unit increase in uric acid, the odds of an increased in OSA risk score is 1.1 or a 10.9 % score increase independent of BMI, age, or gender. It has been previously described an increase in uric acid levels in chronic intermittent hypoxia mainly due to the continuous hypoxia and re-oxygenation cycles (26). Additionally, uric acid increase has been associated with metabolic syndrome which is characterized by an abnormal insulin response (27, 28). It is known that Adenosine in

blood is a sensitive marker of tissue hypoxia, although due to its short half-life in plasma it is not routinely measured. During hypoxia ATP and its precursors degrade releasing purine intermediates and the purine catabolic end product known as uric acid. Previously Saito et al. reported that the index of change in urinary uric acid/creatinine ratio was significantly linked to the plasma level of adenosine in patients with OSA (29). So it is likely that the increase in uric acid levels seen in our study can be associated with hypoxia and re-oxygenation cycles as a consequence of the OSA.

Our study showed that for one unit increase in ALP or GGT, the odds of an increase in OSA risk score is 0.36 % and 0.21 % respectively independent of BMI, age, or gender. Despite these small increases are statistically significant, it is not possible to establish its physiological relevance since very high increases of at least 27 and 45 units will be required respectively to reach just a 10% increase in OSA risk score.

In our study we also found that for each unit increase in hematocrit, the odds of an increase in OSA risk score is 2.5 % independently of BMI, age, or gender. Since it is known that chronic hypoxia is responsible for the increase in hematocrit, mainly due to erythropoietin bone marrow stimulation (30). All these results together allow us to reach the conclusion that in the U.S. population, higher OSA risk scores are associated with an increase in hematocrit, uric acid, and glycohemoglobin.

Since NHANES is a cross sectional survey, it is not possible to determine causality or temporal associations. Another limitations is that despite the high previously reported STOP-Bang questionnaire sensitivity and PPV, the lack of PSG studies in NHANES does not allow to validate the use of this questionnaire in the civilian non-institutionalized U.S. population. In addition since the standard clinical diagnosis of OSA was not done in NHANES this work uses the OSA risk score and not the OSA diagnosis in its conclusions. No mechanistic studies were performed in this work.

This study considered all variables as continues so during statistical analysis none of the biochemistry or physical measurements were categorize as normal or abnormal, which differs from the usual standard of care. A very important contribution of this study is finding that OSA risk is increased in clinically "healthy" population, so increases in hematocrit, uric acid, and/or glycohemoglobin either within normal parameters should be investigated for the possibility of OSA development.

Further studies need to be done before issuing any recommendation for a PSG study or a STOP-Bang score calculation in situations when an increase in hematocrit, uric acid, and/or glycohemoglobin are observed during medical evaluation. Moreover, it needs to be determined if the changes in blood chemistry found in our study are compensatory responses or signs of a very preliminary step in the development of OSA that is still asymptomatic

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APPENDIX

BIOCHEMISTRY

Total Cholesterol was measured using a reaction catalyzed by cholesterol esterase that frees cholesterol and fatty acids. The free cholesterol is oxidized to cholesten-3-one producing hydrogen peroxide. The hydrogen peroxide produced reacts with 4-aminoantipyrine and phenol to produce a colored quinoneimine product. This quinoneimine product's change in absorbance at 520 nm is directly proportional to the concentration of cholesterol in the sample. High Density Lipoproteins (HDL-Cholesterol) were measured directly in serum. Initially apoB containing lipoproteins are excluded from the sample by blocking them with dextran sulfate and becoming no longer reactive for the rest of the assay. The HDL cholesterol is determined enzymatically. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase producing hydrogen peroxide. The hydrogen peroxide produced in the presence of peroxidase reacts with 4-amino-antipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is proportional to the HDL cholesterol concentration and can be measured photometrically. Alanine Aminotransferase (ALT) in plasma was measured using an enzymatic activity rate method. ALT catalyzes the generation of pyruvate which is then reduced with the concurrent oxidation of

NADH to NAD. The rate of this change in absorbance at 340 nm over a fixed-time interval is directly proportional to the ALT activity. Aspartate Aminotransferase (AST) also used an enzymatic activity rate method. AST catalyzes the formation of oxaloacetate, which is then reduced with the concurrent oxidation of NADH to NAD. The rate of change in absorbance at 340 nm over a fixed-time interval is directly proportional to the AST activity. Total Protein was measured using a timed rate biuret method. Proteins in the sample combine with the reagent produce copper-protein chelate. The rate of change in absorbance at 545 nm is directly proportional to the total protein concentration in the sample. Albumin was measured combining it with Bromcresol Purple reagent to form a complex. Changes in absorbance at 600 nm are directly proportional to the concentration of albumin in the sample. Alkaline Phosphatase (ALP) was measured using a 2-Amino-2-Methyl-1-Propanol buffer. ALP catalyzes the hydrolysis of p-Nitrophenylphosphate, into p-Nitrophenol and phosphate. The rate of change in absorbance at 410 nm is directly proportional to the ALP activity in the serum. Gamma-Glutamyl Transferase (GGT) activity was measured from serum using and enzymatic reaction. GGT catalyses the transfer of a gamma-glutamyl to glycylglycine with the production of p-nitroaniline,a colored product. The rate of change in absorbance at 410 nm is directly proportional to the activity of GGT in the serum sample. Bicarbonate was measured indirectly by quantifying the total carbon dioxide (CO2) level in serum or plasma. As CO2 ions diffuse across a membrane the rate of pH change is determined using a pH electrode. The rate of pH change is directly proportional to the CO2 in the sample. Blood Urea Nitrogen (BUN) was measured using an enzymatic conductivity rate method. A volume of sample is injected into the urease reagent in a reaction cup containing an electrode sensitive to changes in solution conductivity. The rate of increase in conductivity is directly proportional to the concentration of urea in the sample. Creatinine was measured using the Jaffe rate method (kinetic alkaline picrate). Sample is combined with alkaline picrate solution. Absorbance readings are taken at both 520 nm and 560 nm. Observed rate measurement is a direct measure of the concentration of the creatinine in the sample. Glucose was measured by the oxygen rate method. A volume of sample is introduced in a reaction cup with an electrode sensitive to oxygen concentration. The rate of oxygen consumption is directly proportional to the concentration of glucose in the sample. Glycohemoglobin was measured directly using HPLC (High-performance liquid chromatography) for fraction separation and the HbA1c fraction flow measured by changes in absorbance at 415 nm which is proportional to its concentration. Lactate Dehydrogenase (LDH) was measured using an enzymatic activity rate method which reduces NAD to NADH. The rate of change in absorbance at 340 nm is directly proportional to the activity of LDH in the sample. Uric acid was measured by oxidation that generates hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and 3, 5-dichloro-2-hydroxybenzene sulfonate in a reaction catalyzed by peroxidase that produces a colored product whose change in absorbance at 520 nm is directly proportional to the concentration of uric acid in the sample. C-Reactive protein (CRP) was measured by latex-enhanced nephelometry. A dilute solution of test sample was mixed with latex particles coated with anti-CRP antibodies, and the formation of antigen antibody complexes quantified with a nephelometer.

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