NHANES 1999-2000 Public Data Release File
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Update: Analytical note was added on Bone Alkaline Phosphatase (BAP) regression equation to compare with 2001-2004 BAP data.

Laboratory 11 – C-reactive protein (CRP), Fibrinogen, Helicobacter pylori, Bone alkaline phosphatase, and Urinary N-teleopeptides (Ntx)

Description

C-reactive protein

C-reactive protein is considered one of the best measures of the acute phase response to an infectious disease or other cause of tissue damage and inflammation. It is used to correct the iron status measures, which are affected by inflammation. It can also be used to measure the body’s response to inflammation from chronic conditions, such as arthritis, and environmental exposures to agents such as tobacco smoke.

Fibrinogen

Fibrinogen is an essential blood-clotting factor and is involved in a range of other functions, including platelet aggregation and smooth muscle proliferation. A growing body of evidence has identified fibrinogen as an important risk factor for cardiovascular disease, the major cause of death in the U.S. The objective of including this measure is to provide data on laboratory, clinical, and socio- demographic correlates of fibrinogen levels. Of particular importance in NHANES, the data will be used to study the relationship between fibrinogen levels and clinically measured lower extremity arterial blood flow as assessed by the Ankle-Brachial Index in the Lower Extremity Disease component.

Helicobacter pylori

This organism has been shown to be the causative agent in chronic-active gastritis, and evidence has almost completely satisfied Koch's postulates for this organisms' pathogenicity in primary duodenal ulcers. More recent evidence has suggested that chronic H. pylori infection as well as early age of H. pylori- acquisition is a critical precursor to gastric carcinoma. Although an explosion of research has occurred over the past decade, many fundamental questions on the route of transmission and the role of environmental risk factors (i.e. food, water) remain to be answered. Because NHANES will have numerous data on environmental exposures in addition to demographic data on participants, these data can be analyzed to add information on potential route of transmission for
this organism.

Bone alkaline phosphatase and Ntx
To assist in the evaluation of skeletal status, two markers of bone turnover are being measured: a) bone alkaline phosphatase, a formative marker in serum, and b) NTX, a resorptive marker in urine.

Eligible Sample

C-reactive protein (CRP) and Helicobacter pylori: Participants aged 3 years and older

Fibrinogen: Participants aged 40 years and older

Bone alkaline phosphatase and Ntx: Participants aged 8 years and older

Data Collection Methods

Blood and urine specimens are processed, stored and shipped to the University of Washington for analysis.

Examination Protocol

Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Vials were stored under appropriate frozen (-20 degrees Centigrade) conditions until they were shipped to University of Washington for testing. The analytical methods are described in the Analytic methodology section.

Analytic Methodology

C-reactive protein
This method quantifies C-reactive protein (CRP) by latex-enhanced nephelometry. Particle-enhanced assays are based on the reaction between a soluble analyte and the corresponding antigen or antibody bound to polystyrene particles. For the quantification of CRP, particles consisting of a polystyrene core and a hydrophilic shell are used in order to link anti-CRP antibodies covalently.
A dilute solution of test sample is mixed with latex particles coated with mouse monoclonal anti-CRP antibodies. CRP present in the test sample will form an antigen-antibody complex with the latex particles.

An automatic blank subtraction is performed. CRP concentrations are calculated by using a calibration curve. Data reduction of the signals is performed by using a storable logit-log function for the calibration curve. These assays are performed on a Behring Nephelometer for quantitative CRP determination.

Fibrinogen
On the STA-Compact, the fibrinogen concentration in plasma is determined quantitatively by the Clauss clotting method. This test method involves

measuring the rate of fibrinogen to fibrin conversion in diluted sample under the influence of excess thrombin. Since under these conditions the fibrinogen content is rate limiting, the clotting time can be used as a measure of the concentration of the fibrinogen and in fact, the clotting time is inversely proportional to the level of fibrinogen in the plasma.

Clot detection by the STA-Compact involves an electromagnetic- mechanical system. The oscillation of a steel ball within the cuvette with the thrombin and diluted plasma is monitored by the STA-Compact. When the oscillation of the steel ball is stopped by clot formation, the sensor registers the time in seconds. The time is translated into fibrinogen concentration from a fibrinogen standard curve, stored on the STA Compact.

Helicobacter pylori
The Wampole Laboratories (Wampole) H. pylori IgG Enzyme-Linked Immunosorbent Assays (ELISA) is intended for the detection and qualitative determination of IgG antibodies to Helicobacter pylori in human serum.

Enzyme linked immunosorbent assays (ELISA) rely on the ability of biological materials, (i.e. antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a participant's serum, antigen-specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase, which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of chromogen substrate tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the participant's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H2SO4, the contentsof the wells turn yellow. The color, which is proportional to the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader. The sensitivity, specificity, and reproducibility of enzyme- linked immunoassays can be comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination, and radioimmunoassays.

Bone alkaline phosphatase
The Tandem-MP Ostase ImmunoEnzymetric Assay is an in vitro device for the quantitative measurement of Skeletal Alkaline Phosphatase (sALP), an indicator of osteoblastic activity, in human serum. This device is intended to be used as an aid in the management of postmenopausal osteoporosis and Paget’s disease.

The Ostase Assay is a solid phase, monoclonal antibody immnumoenzymetric assay. Samples containing sALP are reacted with a solution containing a biotin- labeled, sALP-specific monoclonal antibody. The reaction takes place in a plastic well strip (solid phase) coated with strepavidin and enclosed in a plastic frame. Following the formation of a solid phase/capture antibody/sALP complex, the microplate is washed to remove

unbound sALP and is then incubated with an enzyme substrate. The amount of substrate turnover is determined colorimetrically by measuring the absorbance of the quenched reaction at 405 nm in a microplate reader. The absorbance is proportional to the concentration of sALP present in the test sample. The calculation of sALP concentration in the sample is based on concurrent testing of sALP Calibrators and the Zero Diluent/Calibrator.

The Ostase assay is an in vitro device for the quantitative measurement of skeletal alkaline phosphatase (sALP) in human serum. Changes in sALP have been shown to be useful in participants undergoing therapy for metabolic bone disorders. It may also be an indicator of vitamin D deficiency in some participants.

Ntx (N-teleopeptides)

Osteomark is a competitive inhibition enzyme linked solid-phase immunosorbent assay for the quantitative measurement of the cross-linked N-telopeptides of type I bone collagen (NTx) in human urine. The solid phase consists of microwells onto which cross-linked telopeptides (antigen) is adsorbed. Urine controls, test samples and calibrators are added to the antigen coated 96 well plate. Antibody to the N-telopeptide cross-links that are conjugated to horseradish peroxidase is then added to each well. During an initial incubation period, antigen in the sample competes with the solid phase antigen for binding to the antibody. The wells are then washed to remove unbound material.

Buffered substrate/chromogen reagent is then added to each well. During the final incubation, a blue color will develop when bound antibody-horseradish peroxidase conjugate is present in the well. The color intensity is a measure of the amount of conjugated antibody bound to the solid phase antigen, and is inversely proportional to the amount of antigen in the test sample. The reaction is stopped by the addition of stopping reagent (1N sulfuric acid) which results in a color change from blue to yellow. The absorbance values for the control, calibrators and test samples are determined spectrophotometrically at 450 nm with a 650 nm reference filter, by using a microtiter plate reader.

A standard curve is constructed for each assay by plotting absorbance versus concentration for each calibrator. The antigen concentrations of the samples and control are then read from the curve. Assay values are standardized to an equivalent amount of bone collagen, and are expressed in nanomoles bone collagen equivalents (nM BCE/L) per liter. Often assay results are corrected for urinary dilution by urinary creatinine analysis and expressed in nanomoles bone collagen equivalents per liter (nM BCE/L) per millimole creatinine per liter (mM creatinine/L). This ratio is reported as nM BCE/mM creatinine.

The discovery of urinary cross-linked N-telopeptides of type I collagen (NTx) has provided a specific biochemical marker of human bone resorption. The NTx molecule is specific to bone due to the unique amino acid sequences and orientation of the cross-linked alpha-2 N-telopeptide. Generation of the NTx molecule is mediated by osteoclasts on bone, and is found in the urine as a stable end product of degradation.

Analytic Notes

LBXBAP and URXNT

In 1999-2000 these tests were performed on males 8 years and older and females 18 years and older. Beginning in 2000 these tests are performed on all examinees 8 years and older.

LBXHP1

A value <0.90 is considered negative for the presence of detectable IgG antibody. Values between 0.91-1.09 are considered equivocal and values greater than 1.10 indicates the presence of detectable IgG antibody.

Use of regression equation to trend 1999-2000 Bone Alkaline Phosphatase (BAP) data with 2001-2004 BAP data:

Adjustment of Bone Alkaline Phosphatase was necessary for NHANES 2001 data because of a change of laboratory methods between 2001 (using a Hybritech method) and 2002 (using a Beckman Access method) The distributions of sample person results were compared between NHANES 2001 and NHANES 2002 and the BAP test had significantly ($p < 0.05$) different means. A cross-over study between the two methods was performed to establish regression equations to convert NHANES 2001 values to NHANES 2002 values. The regression equations were applied to the BAP test, and a t test was done after regression that showed no significant differences of BAP test means between the two methods after regression. The regression equations were derived from a spline technique using logarithm-transformed BAP values. The following regression equation was obtained:

$$x = \log(lbxbap)$$
$$lbdbap = \exp(-0.5326 + 1.1139*x - 0.7963*(\max(0, x-4.5151)) + 0.9660*(\max(0, x-4.9030)))$$

where lbxbap is the BAP (ug/L) value from the 1999-2001 Hybritech method and lbdbap is the BAP (ug/L) equivalent value for the 2002-2004 Beckman Access method. The analyst may wish to use this equation to trend the 1999-2004 BAP data.